VOLUME 15 NUMBER 3

NUTRITION REVIEWS

March 1957

Bacterial Synthesis and Destruction of Thiamine by Ryoji Hayashi . . 65

Clinical Nutrition

- Composition of the Diet and Weight Loss . . . 67
- Circulation of Thyroxine in Intestine & Liver . . . 69
- The Action of Vitamin D on Bone Accretion and Resorption . . . 70
- Fat Absorption in Man . . . 71
- Dysproteinemic States in Infants . . . 72
- Intakes of Vitamins A and D . . . 74
- Energy Expenditure and Body Size . . . 76

Animal Nutrition

- Stimulation of Growth by Antibiotics . . . 78
- Manganese Deficiency and Requirement . . . 80
- Assays of Unidentified Chick Growth Factors . . . 82

Experimental Nutrition

- Adequate Versus Optimal Diet . . . 84
- A New Growth Factor . . . 85
- Metabolism of Essential Fatty Acids . . . 86
- Algae as a Protein Supplement . . . 87
- The Regulation of Lipogenesis in Adipose Tissue . . . 88
- Hormonal and Nutritional Factors Influencing Cardiac Glycogen . . . 89
- The Gastric Juice and the Intrinsic Factor of Pernicious Anemia . . . 90
- Calcification of Fetal Bone . . . 92

Notes

- Fat Absorption in the Small Intestine . . . 93
- Calcium Metabolism and Surgical Trauma . . . 94
- Effects of Prolonged Cortisone Therapy on Growth . . . 94
- Cholesterol Absorption in the Rabbit . . . 95
- Clinical Usefulness of Plasma Pepsinogen Assays . . . 95
- Recent Nutrition Books . . . 96

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NUTRITION REVIEWS

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BACTERIAL SYNTHESIS AND DESTRUCTION OF THIAMINE

The presence of a thiamine-inactivating substance in several shellfish and crustacea was noted in Japan by A. Fujita, D. Matsukawa and K. Dohi (*17th Annual Meeting, Jap. Biochem. Soc.*) in 1941. The following year the enzymatic nature of the substance was demonstrated by Fujita and I. Numata (*18th Annual Meeting, Jap. Biochem. Soc., 1942*), who named it aneurinase. K. Shibata and S. Chang (*Vitamins (Japan)* **1,** *209 (1948)*) observed the disappearance of 10 mg. of thiamine introduced by enema into the sigmoidal colon of a patient with habitual constipation and beriberi. They succeeded in recovering from the feces of the patient a potent thiaminase.

A bacterium which secretes a potent thiaminase has been isolated by D. Matsukawa and H. Misawa (*Vitamins (Japan)* **2,** *137 (1949)*) from the feces of a patient with "thiaminase disease." The bacterium was recognized as belonging to a new species of the genus *Bacillus* and was designated as "*Bacillus thiaminolyticus* Matsukawa and Misawa." The second of the "thiaminase bacteria" was isolated from human feces by R. Kimura and S. Aoyama (*Ibid.* **4,** *366 (1951)*), and the third one was also found in human feces by Kimura, T. H. Liao, R. Hayashi, and H. Nakayama (*Ibid.* **5,** *521 (1952)*). The latter bacteria were identified as belonging to new species and named "*Bacillus aneurinolyticus* Kimura and Aoyama" and "*Clostridium thiaminolyticus* Kimura and Liao," respectively.

The incidence of "thiaminase disease" is reported to be 3.1 per cent in Nigata (M. Fujimiya, *Vitamins (Japan)* **3,** *270 (1950)*), 7.1 per cent in Fukuoka (T. Sawada and A. Ichibangase, *Ibid.* **5,** *233 (1952)*) and 3.3 per cent in Kobe (K. Hamada, *Ibid.* **6,** *951 (1953)*).

Other findings suggest that "thiaminase bacteria" are found in the lower intestinal tract in many Japanese people. It is said that about 20 per cent of individuals who yield thiaminase-active broth cultures from feces are suffering from "thiaminase disease" caused by *B. thiaminolyticus* (Matsukawa and Takato, *Vitamins (Japan)* **4,** *380 (1951)*). N. Kobayashi (*Ibid.* **3,** *274 (1950)*) investigated the content of the intestinal tract of 2 patients with "thiaminase disease" caused by *B. thiaminolyticus*, who had died from other causes. He found that the thiaminase was distributed through the entire distal portion of the canal starting at the lower or middle part of the small intestine.

The thiamine level of the blood of patients suffering from "thiaminase disease" caused by *B. thiaminolyticus* is significantly lower than that of normal persons, and thiamine given orally to the patients is actually decomposed by the enzyme in the digestive tract (M. Fujimiya, *Vitamins (Japan)* **3,** *278 (1950)*). When thiamine-deficient diets are fed to these patients, the deficiency symptoms appear in ten days, whereas in normal subjects the same diets must be continued for two to three weeks before the appearance of symptoms (Fujimiya, *Ibid.* **4,** *32 (1951)*). In this connection the observation of K. Takeuchi (*Ibid.* **6,** *269 (1953)*) is interesting. He calculated that between 0.25 and 0.4 mg. of thiamine synthesized by intestinal bacteria is utilized daily by Japanese adults. K. Takato (*Ibid.* **5,** *358, 363, 368, 479, 486 (1952)*) reported an increase in fecal ammonia or related nitrogen compounds of the patient with a resulting acidosis. She believed that the acidosis resulted in a "thiamine leaking," that is, parenteral thiamine was not utilized due to the inhibition of thiamine esterification by the tissues.

There seems to exist some susceptibility to "thiaminase disease" caused by *B. thiaminolyticus*. Y. Horikawa (*Vitamins (Japan)* **4,** *119 (1951)*) observed that among 29 human volunteers who took 4 to 8 mg. of *B. thiaminolyticus* daily with their diets only 2 (one of whom was cured spontaneously in nine days after discontinuance of the oral dose) contracted "thiaminase disease," which manifested itself two or three days after the beginning of the administration.

Using the cup method which is usually applied in the penicillin titer test, K. Hamada (*J. Vitaminology (Japan)* **2,** *67 (1956)*) found that the feces extract from a carrier of *B. thiaminolyticus* is less inhibitory to the growth of this bacillus than that from a healthy subject. He (*Ibid.* **2,** *72 (1956)*) also noted that among the domestic animals, those susceptible to experimental *thiaminolyticus* infection are cats, guinea pigs, white rats and hens. Sheep and rabbits were relatively refractory. The experimentally infected cats and hens contracted "thiaminase disease" and showed beriberi-like symptoms. Hamada (*Ibid.* **2,** *78 (1956)*) believes that the variation among human beings and the differences among the species in their susceptibility to becoming *thiaminolyticus* carriers are related to the amount and type of bile acids in the intestinal tract. The recommended treatment for the disease is the daily administration of 3 g. of homosulfanilamide (Marfanil) for three days, followed by administration of a preparation of lactic acid bacteria to prevent a recurrence of the disease (M. Fujimiya, *Vitamins (Japan)* **4,** *38 (1951)*). In some cases the disease is cured completely by the administration of 0.1 g. of nitrofurfural semicarbazone.

Although a number of cases of thiaminase disease have been associated with *B. thiaminolyticus*, no cases in human beings have been directly attributed to *B. aneurinolyticus* or *Cl. thiaminolyticum*. S. Aoyama (*Acta School Med. Univ. Kyoto* **30,** *127 (1952)*), however, reported that a girl who had beriberi showed almost pure cultures of *B. aneurinolyticus* in her feces; her blood thiamine level was 3.8 micrograms per cent. Thiaminase activity of her feces was not reported. The use of homosulfanilamide eliminated the bacteria from her feces. Thiamine injections added to the regimen resulted in a cure. S. Shintani (*Vitamins (Japan)* **8,** *376 (1955)*) reported a paralytic disease of chickens experimentally attributable to spontaneous infection from *B. aneurinolyticus*.

The thiaminase enzyme in *B. thiaminolyticus* and *Cl. thiaminolyticum* catalyzes the base exchange reaction between the thiazole moiety of thiamine and an amine in the enzyme preparation or in the bacterial cells. The enzyme of *B. aneurinolyticus* catalyzes the hydrolytic split of thiamine, yielding the pyrimidine and thiazole groups. Amines do not accelerate this reaction (R. Kimura *et al.*, *Vitamins (Japan)* **4,** *379 (1951)*; A. Fujita and Y. Nose, *ibid*, **4,** *379 (1951)*).

The physiologic function of bacterial thiaminases has not been clarified. In an earlier editorial on the subject (*Nutrition Reviews* **1,** *403 (1943)*), it was suggested that the thiamine-inactivating factor may be utilized for the synthesis of thiamine. The bacterial cells of *B. thiaminolyticus* (H. Nakayama, *Bull. Yamaguchi Med. School* **1,** *159 (1953)*) and of *B. aneurinolyticus* (T. Kishida, *Vitamins (Japan)* **6,** *423 (1953)*), each grown in a synthetic medium free from thiamine, contained appreciable amounts of this vitamin. It has been found that in a synthetic medium the thiazole and pyrimidine moieties of thiamine promote the growth of *B. thiaminolyticus* (Nakayama, *loc. cit.*) and thiamine, thiamine pyrophosphate, or the pyrimidine group stimulates the growth of *B. thiaminolyticus* (R. Kimura *et al.*, *Vitamins (Japan)* **5,** *248 (1952)*). The enzyme in *B. thiaminolyticus* has been reported to synthesize thiamine from several 5-methylpyrimidyl bases and the thiazole by a base

exchange reaction (A. Fujita *et al.*, *J. Biol. Chem.* **196,** *313 (1952)*). The pyrimidine moiety of thiamine or related pyrimidyl compounds added with or without the thiazole to a synthetic medium was utilized by *B. aneurinolyticus* to synthesize thiamine (H. Ikehata, *Vitamins (Japan)* **7,** *524 (1954)*). Washed cells of *B. thiaminolyticus* and *B. aneurinolyticus* incubated with the pyrimidine and the thiazole moieties synthesized a minute amount of thiamine (R. Hayashi *et al.*, *Ibid.* **7,** *1095 (1954)*). Washed cells of *Micrococcus pyogenes*, which synthesized thiamine from its pyrimidyl and thiazole moieties, had thiaminase activity (Hayashi *et al.*, *Ibid.* **7,** *800 (1954)*).

K. Yonesawa and F. Aoki (*Vitamins (Japan)* **7,** *600 (1954)*) isolated from a human mouth a strain of *Trichosporon*, which had potent intracellular thiaminase. This thiaminase was identified as belonging to the "thiaminase-II" (D. Matsukawa *et al.*, *Ibid.* **7,** *601 (1954)*). Intracellular thiaminases were also reported to be present in several species of *Candida* and related organisms and were extracted from *M. pyogenes*, *E. coli* and several *Bacillus* species (R. Hayashi *et al.*, *Ibid.* **8,** *511 (1955)*).

Evidence is accumulating that the thiaminases are widely distributed among bacteria and related organisms. The thiaminase of bacteria, however, is not important enough to be worthy of nutritional consideration, with the exception of the above-mentioned "thiaminase bacteria," *viz.*, *B. thiaminolyticus*, *B. aneurinolyticus* and *Cl. thiaminolyticum*.

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COMPOSITION OF THE DIET AND WEIGHT LOSS

Various workers (D. M. Lyon and D. M. Dunlop, *Quart. J. Med.* **1,** *331 (1932)*; A. W. Pennington, *Am. J. Digest. Dis.* **22,** *33 (1955)*) have recommended high fat diets in the treatment of obesity. Recently A. Kekwick and G. L. S. Pawan (*Lancet* **II,** *155 (1956)*) have re-examined the possible effect of diet composition as such on weight loss in reducing individuals. The subjects studied were obese, with body weights 35 per cent above standard weight for their heights according to the Metropolitan Life Insurance weight tables.

Before the studies were begun, the subjects were brought into the hospital and placed on diets which allowed free choice of foods. Invariably these individuals lost weight during the first few days on such diets, after which their weight levels stabilized. All studies were carried out after the subjects' weight had become constant.

Six subjects were studied while being maintained on diets in which the proportion of protein, fat, and carbohydrate were kept constant (20, 33 and 47 per cent, respectively) but in which the caloric content varied (2000, 1500, 1000 and 500 calories per day). The water and salt intakes were maintained constant at 3000 ml. of water and 10 gm. of sodium chloride per day. The subjects remained on each of the diets from five to seven days. The diets of differing caloric content were given in a varied sequence. As one would expect, the reduction of caloric intake was followed by a reduction in body weight, with the lowest body weights being attained on the 500 calories intake.

Kekwick and Pawan also carried out nitrogen balance studies with these individuals, and found that they seldom showed negative nitrogen balance even when they were receiving intakes of only 500 calories per day (20 per cent of these being derived from protein). The amount of excreted nitrogen usually paralleled the

intake, with the subjects remaining in approximate equilibrium except when they were on a 2000 calorie intake receiving 100 gm. of protein per day; in this case, the individuals showed positive nitrogen balances. That subjects on reduction diets usually remain in nitrogen equilibrium, had been reported earlier by J. M. Strang, H. B. McClugage and F. A. Evans (*Am. J. Med. Sci.* **181,** *336 (1931)*).

Kekwick and Pawan used the excretion of creatinine in the urine as an index of the breakdown of endogenous protein; they found little or no change in the amount of this compound excreted, indicating that there was no accelerated catabolism of protein tissues. Total body water was determined in some of these obese subjects, using the method of urea dilution described by R. A. McCance and E. M. Widdowson (*Proc. Roy. Soc., London, s. B.* **138,** *115 (1951)*). The initial body water content of 5 obese subjects, expressed as the percentage of body weight, ranged between 50 and 52.5 per cent. After being on the reduction diets for four weeks, the 5 patients lost a total weight of 23.7 kg., but they still had a body water content of 50 to 52 per cent of the reduced body weight. Total water loss during this period had been 11.6 liters of body water. Thus, as they lost body weight, their total body water was also proportionately reduced and about one half or less of the body weight loss was attributable to loss of total body water. In a second study with 4 patients, who were maintained on the 500 calorie diet and showed the greatest individual losses in body weight (2.3 to 3.9 kg. per week), it was found that 50 to 67 per cent of the weight loss was due to loss of fat and only 33 to 50 per cent due to the loss of body water.

Kekwick and Pawan studied the loss of body weights in another series of 14 patients maintained on 1000 calorie diets. These patients also had intakes of 10 gm. of salt and 3000 ml. of water per day. Three different regimens were used. In the first diet, 90 per cent of the calories came from the fat and 6 per cent from protein. In the second diet, 90 per cent of the calories were derived from protein and 8 per cent from fat. In the third diet 90 per cent of the calories were obtained from carbohydrate and 4 per cent from fat. The patients received the three diets in different sequences for approximate periods of seven days per diet.

A rapid loss of weight occurred after feeding the diet which contained 90 per cent of the calories in the form of fat, while the patients who received isocaloric diets where 90 per cent of the calories came from carbohydrate maintained their weights during the brief period of study. The patients who received 90 per cent of their calories in the form of protein showed a positive nitrogen balance of about 10 gm. per day. The individuals who were maintained on either the high carbohydrate or the high fat diet were getting only 1.6 gm. or 2.4 gm. of nitrogen per day, respectively. They showed no large nitrogen deficits and were approximately in nitrogen equilibrium.

The possibility that the large loss of weight on high fat diets was due to loss of fat in the stool and consequent reduction in the effective caloric intakes was ruled out. Fat balance studies showed that the subjects were absorbing 95 to 97 per cent of the ingested fat. Studies of body water content in the patients on the high fat and the high protein diets showed that 41 to 52 per cent of the body weight loss could be attributed to loss of body water and 48 to 59 per cent to loss of body fat.

To determine the relative effects upon weight loss of caloric intake and of the composition of the diet, 5 patients were maintained on a 2000 calorie diet with a normal proportion of protein, fat, and carbohydrate (12, 24 and 64 per cent, respectively). A second ration was prepared with a daily caloric intake of 2600, an in-

creased amount of fat and protein and a decreased proportion of carbohydrate. Four out of the 5 patients studied, after being on the 2000 calorie diet for periods of seven days, lost weight when transferred to the diet with the higher caloric intake, where a higher per cent of the calories was being derived from fat and protein. Thus by increasing the percentage of fat in the diet obese patients appear to lose more weight, even though the caloric content increases slightly.

Interpretation of these findings is obscure. Basal metabolic rates did not appear to be altered by variations in the diet. Increases in insensible water loss on the high fat and the high protein diets suggest to Kekwick and Pawan the possibility of altered metabolic patterns on these diets. Actual explanation of these provocative findings must await further work; it is to be hoped that it will be found possible to use longer experimental periods in future studies.

CIRCULATION OF THYROXINE IN INTESTINE AND LIVER

Although the importance of the liver and gastrointestinal tract in the metabolism of thyroxine was pointed out thirty years ago, systematic investigation of this problem in man has not been undertaken. N. B. Myant (*Clin. Sci.* **15,** *227* (*1956*)) has therefore attempted to document the role of the enterohepatic circulation in the maintenance of the serum thyroxine level.

Myant has studied the biliary excretion of endogenous and exogenous thyroxine labeled with radioactive iodine (I^{131}) in 6 patients with external bile drainage. In 2 of these, the excretion of orally administered thyroxine was measured after restoration of the integrity of the biliary system. A 30-microgram dose of labeled L-thyroxine was given intravenously and the biliary excretion of I^{131} was measured at ten-minute intervals for six hours.

To estimate the clearance rate of endogenous thyroxine, 100 microcuries of I^{131} were injected three days before the clearance measurements. Clearances were estimated as the product of the rate of flow of bile and the bile concentration of I^{131} divided by the plasma I^{131} concentration. These clearance values are minimum estimates since a low value would be obtained if thyroxine were metabolized or stored by the liver, or breakdown products were passed directly to the bloodstream.

Exogenous thyroxine clearance was thus estimated in 4 patients, the values ranging between 13 and 35 ml. per hour. The bile-plasma ratio of I^{131} varied from 1.0 to 1.5 and bile flow fluctuated within 15 and 20 ml. an hour. Fractionation of the bile revealed that 87 to 95 per cent of the I^{131} was recovered in the combined butanol extracts or precipitated with trichloracetic acid. Thus, deiodination and biliary excretion of iodide could not have been more than 10 or 15 per cent of the total radioactivity.

In one of 2 other patients studied endogenous biliary thyroxine clearance was somewhat lower than the above figures. When fecal clearances were measured after establishment of normal biliary drainage they agreed fairly closely with the biliary clearances, suggesting that there was no reabsorption of the excreted thyroxine.

This last observation is somewhat surprising, since thyroxine is obviously absorbed via the gastrointestinal tract. An explanation is suggested by the study of F. N. Briggs, A. Taurog, and I. L. Chaikoff (*Endocrinology* **52,** *559* (*1953*)), who examined the bile from rats that had received I^{131}. The principal I^{131}-labeled fractions in the bile were thyroxine, inorganic iodide and a thyroxine conjugate, probably the glucuronide. When labeled thyroxine was added to bile, and this was placed in the

duodenum, 44 to 68 per cent of the thyroxine was absorbed. However, the labeled thyroxine glucuronide was not so well absorbed. Thus, if a large proportion of the I^{131} in the human bile were present in a thyroxine conjugate, the rate of reabsorption would be greatly decreased.

Briggs and co-workers discussed the importance of the biliary secretion as a possible regulatory mechanism in man. At the clearance rates noted, it was estimated that 0.78 microgram of thyroxine was removed per hour. This is a small factor when compared to the estimated rate of breakdown of thyroxine of 4.0 micrograms per hour.

This investigation is a valuable attempt to assess the importance of the liver and gastrointestinal tract in thyroxine metabolism. Since these studies must be performed on patients with gallbladder disease, and possible concomitant liver disease, the concentration of thyroxine by the liver cannot be assumed to be normal. Also, as noted above, a partial explanation may be given for the failure to reabsorb thyroxine from the gastrointestinal tract, but further fractionation and study of the I^{131}-labeled compounds in the bile is necessary to clear this point.

THE ACTION OF VITAMIN D ON BONE ACCRETION AND RESORPTION

The action of vitamin D in promoting mineralization in rachitic bones is more complex than initial nutritional studies would indicate. Fundamentally any increase or decrease in bone salts represents the difference between the separate rates of accretion of these minerals and their resorption from the bone. An evaluation of these dynamic components of bone metabolism is not possible with the usual balance study techniques, but is approachable with the use of radioactive tracers. A method using radioactive phosphorus has been proposed for this purpose by G. C. H. Bauer, A. Carlsson, and B. Lindquist (*Acta paediat.* **44,** *477 (1955)*).

In brief, the method depends upon the administration of a dose of radiophosphate to an experimental subject and the measuring of the specific activity of the serum and urine inorganic phosphate at frequent intervals. A biopsy of the tibia is obtained at approximately the eighth day and the specific activity of bone phosphate is determined. The location of the biopsy is carefully standardized by means of X-ray and only dense bone is used for determination of the specific activity of the bone inorganic phosphate. In the initial studies a second biopsy was obtained 16 days after P^{32} injection for confirmation of the results.

In order to estimate the rate of bone salt accretion several critical assumptions are made. In the first place, it is assumed that the P^{32} in the biopsy specimen due to simple exchange is very low after 8 days because of the very low P^{32} content of the serum. In the second place it is argued that the bone salt laid down by accretion has not yet been reached by the resorptive process. Therefore, the P^{32} within the bone minerals is equated directly with accretion. The accretion rate, in terms of milligrams of inorganic phosphorus per day per milligram of inorganic phosphorus of bone, is determined from the mean specific activity of the serum inorganic phosphorus, the number of days since the P^{32} administration, and the amount of P^{32} determined in the biopsy sample. The limitations of this approach to bone mineral metabolism have not been clearly established, yet it does provide a research tool for a more penetrating study of bone metabolism. A similar study of bone metabolism has been carried out in rats using Ca^{45} and Na^{22} as tracers.

The disturbances in bone salt metabolism in human rickets have been studied using

the accretion rate method (B. C. H. Bauer, A. Carlsson and B. Lindquist, *Metabolism* **5,** *573 (1956)*). Two infants with vitamin D deficient rickets were found to have lower than normal accretion rates which rose to normal or above with vitamin D administration. As a contrast, two children were studied who had vitamin D resistant rickets. The initial values for the accretion rate of bone phosphorus were roughly comparable to two normal control children of about the same age. Administration of large doses of vitamin D were given and the accretion rates rose markedly to supernormal values.

In commenting upon their observations the authors note that the phosphorus, which is laid down in bone minerals, was of two sources. The first was from the increased retention of dietary phosphorus by decreased fecal and urinary losses, and the second source arises from the mobilization of phosphorus from other parts of the body. The largest store of body phosphorus is within the bone itself. The magnitude of the first source in a ten-month-old infant treated with vitamin D for rickets would be in the order of 0.2 per cent of the bone inorganic phosphorus per day. Because the actual accretion rates observed in the infants treated with vitamin D were more than twice this value the authors concluded that treatment of rachitic children with vitamin D causes not only an increased bone salt accretion rate but also an increased bone salt resorption rate. These speculations require an extrapolation from the observed accretion of the dense bone of a small sample of tibia to the entire skeleton. The authors have already showed that the accretion rate for spongy bone is considerably higher than for compact bone. It would seem reasonable to expect that the difference between total skeleton accretion rate and the phosphorus balance would be even greater than suggested.

It is wise to reserve final opinion concerning the interpretation of the results of Bauer and his co-workers. In the first place the difficulty of this experimental approach has permitted only a limited number of patients to be studied thus far. It will be necessary to extend these observations to establish the range of variation which is normal and the uniformity of the biopsy samples. It is not entirely clear whether the changes in the accretion rate should be regarded as a direct action of vitamin D on bone or whether they are the sequels of the restoration of serum calcium and phosphorus concentrations which are more favorable for calcification. It would seem that the findings in the cases of vitamin D resistant rickets would favor the conclusion that these changes are due to a direct effect of the vitamin on bone. The initial accretion rate was normal in the two patients studied before treatment and higher than normal after treatment. This would be understandable if we were to postulate the defect in this disease to be due to intestinal absorption of calcium and phosphorus whereas the postulated action of vitamin D on the bones still would be operative. The great difference in the ages of the patients with dietary vitamin D deficiency and the patients with vitamin D resistant rickets makes a direct comparison of these groups difficult.

FAT ABSORPTION IN MAN

Opportunities for studying fat absorption in man are uncommon since they depend upon an opportunity to examine thoracic duct chyle. M. J. Albrink, W. W. L. Glenn, J. P. Peters, and E. B. Man (*J. Clin. Invest.* **34,** *1467 (1955)*) were able to perform detailed analyses of the lipids of chyle in 5 patients with thoracic duct fistulas. The emulsified and soluble lipids were analyzed separately, the division being achieved by

slow centrifugation. The concentrations of the lipid components of the creamy layer were calculated as the differences of the values obtained from analyses of the lipid concentrations of the whole fluid and the subnatant following centrifugation.

The lipids of chyle were largely present in the emulsified state. Neutral fat comprised the bulk of the particulate matter, but a small, fairly constant concentration of neutral fat of 6.0 to 10.8 milliequivalents per liter was found in the subnatant. Cholesterol and phospholipids were divided between both fractions and the ratio of free cholesterol to cholesterol ester was similar in both layers.

One patient was studied after periods of fat feeding and fat restriction. Neutral fat in the emulsified layer increased and decreased with the fat content of the diet. This is in agreement with an earlier study of J. P. Peters and E. B. Man (*Metabolism* **2,** *30* (*1953*)) in which it was shown that the lipid pattern of chyle from a patient maintained on intravenous feedings resembled that of serum.

Analyses of the creamy layer disclosed little protein; the amount found was within the error of the method of analysis. Thus, the lipids in this layer are probably in their native state. The cholesterol and phospholipids may be assumed to be dissolved in the neutral fat. In the clear subnatant layer the lipids are held in solution by lipoproteins, most probably synthesized by the liver. When fat is withheld from the diet the chyle and serum lipids are alike, due to the hepatic release of lipoproteins.

In the postabsorptive state the subnatant lipids of chyle vary markedly from those of serum. The cholesterol concentration is lower and neutral fat is higher than in plasma. There is considerable evidence suggesting that a cholesterol-phospholipid-protein complex acts to convey lipids to tissue. The lipids of the subnatant fluid thus reflect the lipoprotein complexes as they are formed and the large difference in neutral fat concentration may be attributed to rapid utilization by the peripheral tissues. These findings in man have essentially confirmed the earlier similar hypotheses based upon experimental studies with animals.

DYSPROTEINEMIC STATES IN INFANTS

During the past year 3 groups of workers have reported results of studies of series of infants with hypochromic anemia whose clinical appearance was differentiated from that large group of anemic infants by the presence of edema, hypoalbuminemia, and low concentrations of copper in the serum. In one such study by R. A. Ulstrom, N. J. Smith, and E. M. Heimlich (*Am. J. Dis. Child.* **92,** *219* (*1956*)) detailed reports on 4 infants are given. These infants were all under 7 months of age. In one of the patients the hemoglobin concentration was only minimally reduced at the time that the edema appeared, and the anemia became rapidly severe during the period of observation. These infants were all of normal birth weight and had ingested an optimal diet. There was no evidence of renal or hepatic disease. Marked hypoproteinemia was present in each patient at some time during the illness. The hypoproteinemia was characterized by a uniform reduction in the concentration of albumin and all globulin components.

Continued normal growth rates in all patients, as well as the nitrogen balance studies performed in two patients, indicated that the overall nitrogen balance was positive. However the nitrogen excreted by the kidneys was high in comparison with the dietary intake of protein. Anemia was present, or developed, in each of the patients. The anemia was microcytic and

hypochromic. Hypoferremia, hypocupremia, and a reduction in the total iron-binding capacity of the plasma were found. There was elevation of the free erythrocyte protoporphyrins. Bone marrow aspiration failed to reveal the normoblastic hyperplasia characteristic of simple iron deficiency anemia. Intestinal absorption of iron was within normal limits as demonstrated by an iron balance study using Fe^{59}-labeled eggs as a dietary source of iron.

Studies of plasma protein turnover rates were made in 3 of the infants using tracer doses of phenylalanine 3-C^{14}. A comparison with studies taken on 2 normal infants showed that the rate of incorporation of this isotope into each protein fraction was similar in the hypoproteinemic and the normal infants. The isotope label disappeared from the circulating protein at an increased rate in the infants with hypoproteinemia. This finding was most striking in the albumin fraction. These authors concluded that this increased rate of plasma protein degradation and the failure to compensate for it by an increased rate of production was responsible for the low circulating levels of albumin and globulin in these infants. Although gamma globulin could not be demonstrated in the plasma by Tiselius or paper strip electrophoresis, 1 of the infants was able to respond to antigenic stimulation with the production of antibodies measurable by immunologic techniques. The number of normal plasma cells in the bone marrow aspirates was within normal limits. The plasma albumin of 2 of the patients was antigenically similar to that of normal infants and adults by the Ouchterlony agar plate precipitin technique.

Ulstrom, Smith, and Heimlich have pointed out that this syndrome developed in normal infants on a normal diet and appeared to be transitory in that the symptoms and abnormal laboratory findings disappeared spontaneously after a period of about two months. Although they did not feel that the anemia was due to iron depletion, the hemoglobin levels rose in each patient following the administration of medicinal iron. No attempt was made in this particular study to evaluate the role of medicinal copper in the treatment of these patients.

In the same issue of this journal (*Ibid.* **92,** *254* (*1956*)) P. Sturgeon and C. Brubaker present somewhat similar findings in a group of 5 infants which they have interpreted as copper deficiency. Four of their patients ranged in age from 6½ to 9 months and their fifth case was 2½ years old. Their patients all had moderately severe anemia with hemoglobin concentrations of less than 7.5 g. per 100 ml., microcytosis, hypochromia and profound reduction in the concentration of serum iron. Although the serum total iron-binding capacity was also slightly reduced, the per cent saturation was in the range commonly seen in typical cases of iron deficiency anemia. The serum protein concentration was similarly reduced in all cases. The reduction in the albumin fraction was greater than that in the globulin fractions, but they found that the greatest globulin decrease was in gamma globulin and that changes in alpha 1, alpha 2, and possibly the beta fractions were not distinctly evident. They found a greatly reduced concentration of serum copper (24 to 68 mcg. per 100 ml.). In contradistinction to typical cases of iron deficiency anemia, in which an increased serum copper concentration is usually found, this reduction was quite striking. In their experience the average concentration of serum copper at 8 months of age was approximately 140 micrograms per 100 ml., ranging from 85 to 200 micrograms per 100 ml.

Sturgeon and Brubaker have presented the following reasons for their interpretation that the low serum copper levels in their patients might represent actual depletion of total body copper. Hypocupremia appears in copper deficient animals. A rise in serum copper concentration occurred

following its oral administration in 2 cases and after dietary changes in the remaining 3 cases. The occurrence of hypocupremia in infants manifesting iron deficiency, and possibly protein deficiency of dietary origin as well, was compatible with this hypothesis. Their infants subsisted almost exclusively on milk, a food known to be an extremely poor source of copper. However they do point out that their studies are not adequate to demonstrate whether the anemia and/or the hypoproteinemia could be corrected independently of the hypocupremia.

The possible role of modern food processing and the general availability of milk, which has been produced without access to copper at any stage of its processing, is seriously considered. Alternate hypotheses of multiple nutritional deficiency and the presence of a single deficient mechanism possibly involving copper metabolism were put forward.

M. E. Lahey and W. K. Shubert (*Society for Pediatric Research, May 8, 1956*) have presented observations on another group of 5 infants ranging in age from 6 to 15 months. These infants were all on diets consisting exclusively of milk. They all had hypoferremia, hypocupremia, and very low saturation of the iron-binding protein in their serum. In one of their patients, Lahey and Shubert were able to demonstrate a secondary reticulocyte response to copper following a partial response to oral iron therapy.

There can be no doubt that the concomitant occurrence of hypoproteinemia, hypocupremia, and hypoferremia with greatly reduced saturation of the iron-binding protein constitutes a newly recognized syndrome in infants. However, interpretation of these findings as manifestations of a simple dietary copper deficiency would seem unwarranted at the present time. The reduction in the serum globulins generally may well involve reduction in the amount of ceruloplasmin (the copper-binding protein) which in itself may be the explanation for the low serum copper concentration. Ceruloplasmin exists in the alpha-2 globulin fraction. The occurrence of this syndrome in very young infants who are fed optimal diets would again suggest that a dietary copper insufficiency might not be adequate to explain the occurrence of all instances of this syndrome unless their body stores of copper were greatly depleted at the time of birth. Certainly confirmation of the observations of Lahey and Shubert, demonstrating a definite therapeutic response to copper in infants maintained on a milk diet who failed to respond completely to iron, is badly needed.

Finally no experimental data showing a reduced life of the serum protein in copper deficient animals are available, and in this respect the studies of Ulstrom, Smith, and Heimlich are unique. Many of these infants have agammaglobulinemia without recurrent infections and the inability to manufacture antibodies. In some ways these observations of dysproteinemia fit in rather well with the spectrum of conditions having an associated agammaglobulinemia recently reviewed by R. A. Good (*Pediatrics* **18,** *109 (1956)*).

INTAKES OF VITAMINS A AND D

In a recent review (*Nutrition Reviews* **14,** *338 (1956)*) the alarming rise and widespread incidence was noted of so-called "idiopathic hypercalcemia" in infants in various parts of Europe and the United Kingdom. The most likely cause of the disease appears to be high levels of vitamin D intake, coupled with a high calcium intake. The report upon the intake of vitamin A and D and ascorbic acid of a group of

U. S. children who were studied for the first five years of life (V. A. Beal, *J. Nutrition* **60,** *335* (*1956*)) is thus pertinent and of general interest.

The paper represents one of a series (V. A. Beal, *J. Nutrition* **50,** *223* (*1953*); *Ibid.* **53,** *499* (*1954*); *Ibid.* **57,** *183* (*1955*)) in which the results are recorded of repeated studies upon normal children living at home made during the first five years of life. Data upon 30 boys and 34 girls from upper middle class families in the Denver area are presented. The median intake of vitamin A from all sources was found to be approximately 1,000 I.U. per day during the first month of life and rose rapidly to 10,000 I.U. per day at approximately 1 year of age. It then fell to approximately 6,000 I.U. at 2 years of age and remained at about this level of intake. The highest levels of intake found after the first month of life varied between 12,000 and 25,000 I.U. per day and the lowest levels varied from 1,300 to 5,000 I.U. The average child received vitamin concentrate 64 per cent of the time. Between 1946 and 1949, 62 per cent were given concentrates containing 10,000 I.U., or more, per 10-drop dose. Since 1950 only 8 per cent received such high potency material and most received concentrates containing 5,000 I.U. per 10-drop dose, mostly as multi-vitamin preparations.

The median vitamin D intake during the first month of life was found to be 225 I.U. This rose to about 1,000 I.U. at 6 months of age and then gradually fell to an intake of approximately 400 I.U. per day. Throughout the first five years of life the highest intakes varied from 1,000 to 2,700 I.U. per day. From the previous study (Beal, *Ibid.* **53,** *499* (*1954*)) the median calcium intake was shown to rise from about 600 mg. at 1 month of age to slightly over a gram at 6 months of age. It then declined to a low of 800 mg. at 2 to 3 years of age before rising to approximately a gram at 5 years of age. The highest intakes encountered varied between 1.0 and 1.8 g. per day during the five years.

All of the children received some vitamin D during the first three months of life, but after that time no intake of vitamin D was recorded for some at each age. Whether this means that some of the children never received vitamin D after the first three months of life, or whether the intake was irregular, is not clear from the paper. In any event, many of the children had intakes of vitamin D below 50 I.U. between the second and fifth years. Subsequent reports from the Child Research Council upon the growth and development of these children will be of obvious interest and importance.

The ascorbic acid intakes are perhaps less remarkable. Although 0 intakes are recorded during the first year of age, after this period at least 75 per cent of the children had intakes over 40 mg. per day. The highest levels of intake vary from 150 to 200 mg. per day. Since most of the children apparently receive adequate amounts, these data also will be of more interest when the individual intakes are related to the development of the children.

Although it has been pointed out that hypercalcemia in infants in the United States and Canada are apparently rare for unknown reasons, the high intakes recorded here for vitamins A and D are reason for concern. P. C. Jeans and G. Stearns (*J. Pediat.* **13,** *730* (*1938*)) have indicated that intakes of vitamin D above 1,500 I.U. per day are probably detrimental; this is also indicated by the current studies on hypercalcemias. H. G. Morgan *et al.* (*Lancet* **270,** *925* (*1956*)) raise the question whether or not "more illness may now be due to vitamin D than the rickets it is given to prevent." The long-term effects of moderately high intakes of the essential nutrients clearly deserve more attention and critical study.

ENERGY EXPENDITURE AND BODY SIZE

Interest in the relation of basal metabolic rate and body size, after suffering a series of fashionable fluctuations, has been revived in terms of the concept of lean body mass, or fat-free body mass (*Nutrition Reviews* **11,** *301* (*1953*)). At the same time interest in the classical controversy over units of expression of metabolism, the significance of theoretical body surface, and the geometrical similitude of mammalia has also been revived. The classical basis of the so-called "surface law" was severely damaged by M. Kleiber (*Physiol. Rev.* **27,** *511* (*1947*)) who inclined to the acceptance of the empirically fitted unit $W^{3}/^{4}$.

W. von Döbeln (*Acta physiol. Scand.* **37,** *suppl. 126* (*1956*)) has approached this subject in terms both of the basal and of the maximal metabolism in man. He used 35 adult males and 35 adult females, of a height and weight which indicated approximate anthropometric correspondence with previous Scandinavian studies (B. Broman, G. Dahlberg, and A. Lichtenstein, *Acta paediat.* **30,** *1* (*1942*)). Having measured body density by the immersion method, he also gives a detailed description of the apparatus and method, and a detailed analysis of the sources and magnitudes of error due to air inclusion.

He measured the basal or standard metabolic rate by the Douglas bag method and Haldane gas analysis, on a total volume of expired air of approximately 100 liters, which represents a time interval of approximately 10 to 15 minutes. The rates for males and females, respectively, were 269 ± 4.72 and 226 ± 3.32 ml. of oxygen per minute. This rate was higher for males than for females if expressed per individual, per kilogram, or per square meter of body surface. Per kilogram of fat-free body mass, the rate for females was slightly higher. Per kilogram of fat-free tissue to the ⅔ power, the males showed a slightly higher rate. On the basis of this close approximation of male and female rates when expressed in the last way, and on the theoretical dimensional basis of the relation, within species, of the cross-sectional area of the aorta and of the trachea to the ⅔ power of body weight (G. Dreyer, W. Ray, and E. W. A. Walker, *Proc. Roy. Soc. s.B.* **86,** *56* (*1912*)) (as these dimensions can be said ultimately to limit the metabolic rate) von Döbeln concludes that this method of expression should be used for basal metabolic rates.

Maximal metabolic rates were derived using the method of P. O. Astrand and I. Rhyming (*J. Applied Physiol.* **7,** *218* (*1954*)) by use of pulse rates and by extrapolation from submaximal rates. This approach makes a great many large assumptions. A major methodological criticism is that the work periods were of six minutes and no adequate account appears to have been taken of the possibility of an oxygen debt having been incurred. For males and females, respectively, the rates computed were 3.91 ± 0.09 and 3.06 ± 0.09 liters of oxygen per minute. In finding a basis for expression of maximal metabolic rate it is assumed that the non-fatty cytoplasm of adipose cells does not participate in the increased work above basal, and so it has been removed. This is a broad assumption which may be valid, but it would be equally valid to propose that the gut, the brain, and a number of other parts of the body do not contribute to the increased work of a group of muscles. Using this rather artificial basis the author finds the maximal rates for males and females to be 263.2 ± 4.50 and 248.9 ± 7.13. The difference is not statistically significant, but it is regarded as suggestive, and the author proposes that any real difference might be explained by the difference between male and female hemoglobin content. The difference be-

tween male and female hemoglobin levels, however, is significant and although the levels of both sexes can be raised by prolonged treatment with iron, it does not appear to be possible to diminish the sex difference in level (R. C. Garry, A. W. Sloan, J. B. de V. Weir and M. Wishart, *Brit. J. Nutrition* **8,** *253 (1954)*).

Dr. von Döbeln also proposes that the maximal work rate of the organism is determined by the maximal work rate of the heart. It is possible to calculate that the work of the heart is, under certain circumstances, a ⅔ power function of the minute volume which in turn can be assumed to be proportional to the mass of working tissue. Thus it can be concluded from this argument that the best basis of expression of the maximal rate is the ⅔ power of the mass of working tissue. All the many assumptions made in this calculation are susceptible to serious criticism, but the approach to the problem is illuminating and handled well.

M. Kleiber, A. H. Smith and T. N. Chernikoff (*Am. J. Physiol.* **186,** *9 (1956)*) and M. C. Conrad and A. T. Miller (*Am. J. Physiol.* **186,** *207 (1956)*) have attempted to codify the relation between metabolic rate and body size of rats. Both groups have, in this instance, been particularly concerned with the effect of age on the general metabolism-body-size relationship. Both also have attempted to measure basal metabolism at an arbitrary thermal neutrality. Kleiber and his group chose 30°C. and Conrad and Miller 28°C. In considering work of this sort with small untrained animals, it must be borne in mind that basal or even post-absorptive conditions, if meaningful at all, are of very doubtful comparability with corresponding conditions in man, and the performance of the estimations at thermal neutrality does not necessarily improve the situation if the usual living conditions of the rats are 24°C. or 25°C.

Kleiber *et al.* have made a large number of three-hour determinations of oxygen and carbon-dioxide on female rats from birth to one thousand days of age, which approximates 6.5 g. to 350 g. There is a minor error in the table for the thirty-six-day group. They have presented a series of regression equations to describe the data, and they find the effect of age to have three phases: Up to about forty to seventy days the metabolic rate per kilogram to the ¾ power increases; between seventy and two hundred to three hundred days it decreases; thereafter it slowly increases again. The work is a valuable addition to the sum of information on the metabolic rate of rats, but the teleological discussion, thinly disguised as physiology, is unhelpful.

Conrad and Miller, dealing with essentially the same problem, cover a less ambitious age range of twenty to one hundred eighty days. In addition they made body analyses in an attempt to determine if any better unit of body size could be used. They found some confirmation of a dip in metabolic rate per kilogram between twenty and thirty days previously shown by B. Grad (*Am. J. Physiol.* **174,** *481 (1953)*). They do not hazard a guess as to its origin. It is probably associated with the declines in body weight and food intake which normally occur in the immediate post-weaning period. The data of Kleiber *et al.* (above) do not show any evidence of this dip, but Kleiber's values are true indirect calorimetric values, while Grad and Conrad and Miller only recorded oxygen consumption.

They found the expression of body size which gave the nearest approach to linearity of their data to be kilograms to the ½ power. Calculation from the data given (with means at thirty, sixty and one hundred twenty days) indicates that although the use of fat-free body mass does not make the metabolic rate a constant at different ages it does make the sixty and one hun-

dred twenty day rates rather closer. Neither individual values nor measures of dispersion are given, however. The authors use the definition of A. Keys *et al.* (*The Biology of Human Starvation*, (*1950*)) for active tissue mass, that is weight − (fat + extracellular fluid + ash). The assumption that the extra-cellular fluid, or other tissue components as assumed by von Döbeln, is sufficiently metabolically inert to be disregarded requires a great deal more critical examination than it has so far received.

STIMULATION OF GROWTH BY ANTIBIOTICS

The function of antibiotics as growth stimulants for animals continues to absorb the interest of a number of researchers (*Nutrition Reviews* **12,** *92, 152, 287* (*1954*); **13,** *89, 117, 169* (*1955*)).

A recent study at Kansas State College, Manhattan, Kansas, suggests that at least a part of the effect of antibiotics upon growth is due to their effect on the physiology of bacteria. The result of this effect is to make the bacteria more susceptible to the host defense mechanisms, and thereby less detrimental to the body (J. J. Radisson, E. E. Bartley, T. H. Lord and M. J. Swenson, *J. Dairy Sci.* **39,** *1386* (*1956*)). In this study, salmonella species and coliform bacilli were isolated from the feces of calves with, and without, aureomycin. The aureomycin was administered to 4 treatment groups as follows: 45 mg. daily per calf by capsule, 45 mg. daily in milk, 125 mg. weekly per calf by capsule, and 1 per cent of an aureomycin concentrate (containing 3.6 g. aureomycin per pound) in calf starter.

The sensitivity of the isolated organisms to phagocytosis *in vitro* was tested by using leucocytic suspensions obtained from the blood of both aureomycin-administered calves and control calves. Also, the effect of aureomycin on the phagocytic activity of leucocytes was determined by testing the sensitivity of a number of bacterial species to leucocytes obtained from the blood of control calves and aureomycin-administered calves.

The bacterial isolates were standardized by dilution with saline to a concentration of 4×10^8 bacteria per ml. Leucocytes, separated by centrifuging, were taken up in blood plasma to a standard concentration of 40,000 per ml.

The bacterial and leucocyte suspensions were combined by pipetting ¼ ml. of each into a sterile tube. The tubes were then incubated for thirty minutes at 38°C.

Following incubation, the phagocytic index was determined by examining microscopically the number of bacteria ingested per leucocyte. The term "phagocytic index" refers to the number of bacteria ingested per 100 leucocytes.

The isolates were made at alternate weeks through seven weeks. The results indicated that coliform bacteria from antibiotic-fed calves were significantly more sensitive to phagocytosis than were similar organisms from control calves. A similar effect, approaching significance, was noted with salmonella. The average coliform phagocytic index for control-calf leucocytes was 12.8 (37 cultures tested) contrasted with an index of 20.1 (49 cultures tested) for calves receiving 45 mg. or more, daily, of aureomycin from birth.

Although ingestion of aureomycin increased the sensitivity of intestinal bacteria to phagocytosis, it had no influence on the phagocytic activity of the leucocyte. Leucocyte from control calves apparently possessed phagocytic activity equal to that from animals fed aureomycin.

These results lend support to the view that antibiotics may stimulate growth in young ruminants by modifying the virulence of

intestinal bacteria, particularly in early life when the phagocytic activity of leucocytes is low.

The composition of the increase in gain in body weight from antibiotic feeding has been investigated by workers at the University of Delaware (D. L. MacFadden and C. R. Richards, *J. Dairy Sci.* **39,** *1438 (1956)*).

Antipyrine was employed in a dilution technique as developed by H. F. Kraybill, O. G. Hankins and H. L. Bitter (*J. Applied Physiol.* **3,** *681 (1951)*) to determine total body water at 1, 4, 8, 12 and 16 weeks of age in control calves, and in calves receiving 50 mg. of aureomycin daily.

The 7 aureomycin-fed calves gained weight at a significantly greater rate than the 7 control calves, the aureomycin-fed calves averaging 252.8 pounds in body weight at age 16 weeks, as compared with an average body weight of 217.7 for the control calves.

Total body water, estimated from the dilution of antipyrine injected into the circulation, was consistently higher in calves receiving the antibiotic. The mean body water value was 73.08 per cent at 16 weeks of age, as compared with an average body water value of 70.36 per cent in control calves.

Since there is an inverse relationship between body water and body fat, the calculated body fat was consistently lower in the antibiotic-fed calves when compared with the control calves.

These results are somewhat at variance with those of Rusoff and associates (*J. Dairy Sci.* **37,** *488 (1954)*) who noted a higher percentage of fat in a representative meat cut (the twelfth rib) of antibiotic-fed calves than in control calves. Increase in back-fat thickness in swine from aureomycin feeding has also been noted (T. W. Perry, W. M. Beeson and B. W. Vosteen, *J. Animal Sci.* **12,** *310 (1953)*).

In view of the limited data thus far available on the application of indirect methods for body water determination in ruminants, it is unfortunate that MacFadden and Richards did not support their estimated body composition data with actual measurements for fat and water content in slaughter material. This would appear to be particularly desirable in view of the apparently conflicting results that have been published with reference to the composition of animal weight gain as influenced by antibiotic feeding.

Several reports have indicated that antibiotic feeding in animals results in a significant increase in the retention of dietary calcium (*Nutrition Reviews* **12,** *336 (1954)*).

This postulate has been tested recently in some carefully conducted experiments with rats (E. W. Hartsook, *J. Nutrition* **60,** *97, (1956)*). Weanling rats were fed a purified diet of known composition with and without added calcium, and with and without added aureomycin. Increasing the dietary calcium from 0.29 per cent to 0.55 per cent resulted in a highly significant increase in carcass calcium gain (22.5 per cent). Aureomycin supplementation of the basal diet, or of the basal diet with added calcium, did not influence the retention of calcium. The aureomycin supplement did, however, increase the carcass fat gain (ether-extract gain) by 10.7 per cent over that of the control rats. Carcass dry matter gain was significantly decreased by antibiotic feeding.

Since carcass water was decreased with antibiotic feeding, and body fat was increased, these carefully determined quantitative data appear to be the reverse of those noted by MacFadden and Richards in calves as determined by indirect means.

One must, however, question the value of data obtained with the rat in attempting to establish antibiotic function in the stimulation of animal growth. This species does not exhibit growth response to antibiotic feeding, as Hartsook again confirmed in his recent carefully controlled studies.

MANGANESE DEFICIENCY AND REQUIREMENT

The essentiality of manganese in trace amounts for the rat, rabbit, mouse, and chick has been considered in earlier reviews (*Nutrition Reviews* **1,** *394* (*1943*); **2,** *50* (*1944*); **3,** *174* (*1945*); **4,** *227* (*1946*)). It is to be expected that manganese is a dietary essential for all higher animals, including man, because of its indispensable role in various enzymatic reactions which have been demonstrated *in vitro*. However, few studies with higher animals have been reported, and no studies have yet shown, that man requires manganese (*see* E. J. Underwood, "Trace Elements in Human and Animal Nutrition", *Academic Press Inc., New York, Ch. 8, 233* (*1956*)).

Manganese deficiency in laboratory animals is known to cause poor growth, decline in reproductive capacity, faulty bone growth, and nervous disorders. Recent studies have extended observations of manganese deficiency to other animal species.

Manganese Deficiency in Swine: Studies of manganese deficiency in swine have been carried out by M. P. Plumlee *et al.* (*J. Animal Sci.* **15,** *352* (*1956*)). Four experiments were designed to study the effect of manganese on growth, fattening, and reproduction. The manganese-low basal rations, semi-purified, contained from 0.5 to 3.5 mg. of manganese per kilogram of diet. In the first 3 experiments weanling pigs weighing between 30 and 43 pounds were fed the various manganese-low diets. No effects of manganese upon growth or reproduction were observed.

The fourth experiment was made with 10 crossbred suckling female pigs about 3 weeks of age, with an average weight of 9.4 pounds. They were fed a glucose-dried skim milk diet containing only 0.5 mg. of manganese per kilogram of diet as determined by analysis. Ten control pigs were fed the same diet supplemented with 40 mg. of manganese per kilogram of diet.

Under these conditions a manganese deficiency was obtained in the pigs fed the basal diet, beginning when they weighed 55 to 60 pounds. They developed the following symptoms: (a) reduced skeletal growth, shortening of the leg bones, enlarged hocks, and an accompanying skeletal or muscular weakness; (b) increased back fat thickness; (c) irregular estrus cycles or, in some instances, complete lack of estrus; (d) resorption of fetuses, or birth of small, weak, ataxic pigs which could neither stand nor walk properly; (e) poor udder development in the sow and almost complete absence of milk production and secretion; (f) a reduction in the level of manganese in the tissues of the sows as well as in their milk and offspring. None of the signs of deficiency was observed in the control group supplied with manganese in the diet.

The finding of increased fat deposition in manganese-deficient pigs is deserving of further study. The deficient pigs were obviously stunted as far as skeletal growth was concerned but had so much extra body fat that their weight was similar to that of the control animals throughout the growing period.

The occurrence of leg weakness in manganese-deficient pigs is probably related to perosis, or slipped tendon, which occurs in manganese-deficient chickens, and to the shortened bones seen in deficient rats and rabbits. A leg weakness in swine fed manganese-low diets has been previously reported by R. C. Miller, T. B. Keith, M. A. McCarty, and W. T. S. Thorp (*Proc. Soc. Exp. Biol. Med.* **45,** *50* (*1940*)) and by Keith, Miller, Thorp, and McCarty (*J. Animal Sci.* **1,** *120* (*1942*)).

The minimum requirement of manganese cannot be determined from the work of Plumlee *et al.* (*loc. cit.*). Miller and coworkers (*loc. cit.*) and R. H. Grummer, O. G. Bentley, P. H. Phillips, and G. Bohstedt (*J. Animal Sci.* **9,** *170* (*1950*)) re-

ported that 11 to 14 mg. of manganese per kilogram of diet was not sufficient for pigs. On the other hand, S. R. Johnson (*Ibid.* **2,** *14* (*1943*); **3,** *136* (*1944*)) and Plumlee and co-workers (*loc. cit.*) could not find signs of manganese deficiency in weanling pigs receiving 0.5 mg. and 3.5 mg. of manganese, respectively, per kilogram of diet. It is probable that as yet unknown conditions can affect the manganese requirement. The age of the pig at start of experiment, manganese stores in the tissues, and the levels of calcium and phosphorus in the ration are undoubtedly important factors.

Manganese Deficiency in the Rat: The symptoms of manganese deficiency in the pig reported by Plumlee and co-workers (*loc. cit.*) were very similar to those seen in the rat by R. M. Hill, D. E. Holtkamp, A. R. Buchanan, and E. K. Rutledge (*J. Nutrition* **41,** *359* (*1950*)). These workers noted an ataxic condition and loss of equilibrium in the offspring of manganese-deficient rats fed reconstituted milk supplemented with copper, iron, and necessary vitamins.

Bone changes, such as decreased length, breaking strength, and density, have been seen in manganese-deficient rats (M. O. Amdur, L. C. Norris, and G. F. Heuser, *Proc. Soc. Exp. Biol. Med.* **59,** *254* (*1945*)) and in the rabbit and chicken (*see Nutrition Reviews* **3,** *174* (*1945*); G. H. Ellis, S. E. Smith, and E. M. Gates, *J. Nutrition* **34,** *21* (*1947*)). These latter workers also found lowered liver arginase activity and lowered phosphatase of the bone in the deficient animals.

The optimum manganese intake for growth of white rats was reported to be about 2 mg. per rat per day at 30 days of age and about 3.75 mg. per day at 50 days of age, according to Holtkamp and Hill (*J. Nutrition* **41,** *307* (*1950*)). Such values would be equivalent to at least 300 mg. of manganese per kilogram of diet, which are values much higher than are usually given to rats. Data presented by B. M. Anderson and H. E. Parker (*Ibid.* **57,** *55* (*1955*)), indicate that at least 50 mg. of manganese per kilogram of diet may be required by the rat for optimum gains. These levels are unusually high and require confirmation before they can be accepted. They may be the result of mineral imbalances in the diet (*see Nutrition Reviews* **9,** *135* (*1951*)).

Manganese Deficiency in Fowl: Manganese deficiency in the duck has been studied by R. Bernard and J. M. Demers (*Revue canad. biol.* **11,** *147* (*1952*)) who have reported symptoms of poor growth, perosis, decreased manganese content of bones, and lowered alkaline phosphatase of the serum. More recent work on manganese in the duck has been made by R. Van Reen and P. B. Pearson (*J. Nutrition* **55,** *225* (*1955*)). Day-old white Pekin ducklings were fed synthetic diets containing from 0 to 78 mg. of manganese per kilogram of diet for as long as 3 weeks. It was found that at least 40 mg. of manganese per kilogram of diet was needed to support good growth and to prevent perosis (slipped tendon). In liver, kidney, heart, and plasma of the deficient animal there were reduced amounts of alkaline phosphatase activity. No changes were seen in levels of cytochrome oxidase, catalase, diphosphopyridine nucleotidase (DPNase), or isocitric dehydrogenase in the liver. Also the authors found no significant changes in the riboflavin, niacin, or pantothenic acid content of the liver.

J. R. Couch, L. E. James, and R. M. Sherwood (*Poultry Sci.* **26,** *30* (*1947*)) stated that hens in the second year of production required about 70 mg. of manganese per kilogram of diet, whereas pullets did not require over 41 mg. for egg production.

Manganese Deficiency in Cattle: G. E. Hawkins, Jr. *et al.* (*J. Dairy Sci.* **38,** *536* (*1955*)) have studied manganese and calcium and phosphorus interrelationships in calves

using supplemented milk diets containing less than 1 mg. of manganese per kilogram. Although it was evident that other deficiencies may have been present in the basal diet, additions of manganese did not increase growth except in the presence in the diet of 3 to 5 per cent of monocalcium phosphate.

O. G. Bentley and P. H. Phillips (*J. Dairy Sci.* **34,** *396 1951*)), have fed dairy cows natural rations consisting of manganese-low hay plus corn silage and a grain mixture of corn and corn products, which contained an over-all manganese content of 7 to 10 mg. per kilogram of diet. Cows fed this diet were slow to exhibit signs of estrus and had a greater number of calves with weak legs. These difficulties were prevented by manganese supplementation.

Studies in 1953 in Holland by J. Grashuis, J. J. Lehr, L. L. E. Beuvery, and A. Beuvery-Asman (as quoted by Underwood, *loc. cit.*) also indicated that manganese deficiency can exist under natural conditions. Grashuis and co-workers found poor growth, leg deformities, poor fertility, and frequent abortion in cattle grazing on plants which were grown on certain sand and peat soils low in available manganese. The disorders were prevented by 2 g. of manganese per day per cow.

The above studies demonstrate the essential nature of dietary manganese for growth, reproduction, and the prevention of nervous and bone disorders in a wide variety of animals. Little agreement exists on actual minimum requirements, which suggests that further work is needed.

It appears quite definite that more attention should be given to possible manganese deficiencies in domestic animal species, and in man under natural conditions. Attention to this problem is needed because of the indispensable role of manganese in metabolism and due to its very limited distribution (or poor availability) in milk, in certain pastures, and in certain other foods, such as corn, meat, fish, and sea foods. Additional studies on the relationship of manganese to other nutrients are also needed.

ASSAYS OF UNIDENTIFIED CHICK GROWTH FACTORS

It is known that several unidentified organic growth factors for chicks exist in a variety of crude products (*see Nutrition Reviews* **14,** *75, 179 (1956)*). Although progress has been made on the isolation of these factors, improved assay procedures are needed to obtain consistent and significant differences in growth.

Recent work indicates some of the difficulties associated with the studies on unidentified growth factors and offers suggestions as to how these difficulties may be overcome. B. D. Barnett and H. R. Bird (*Poultry Sci.* **35,** *705 (1956)*) have reported that in 1953 responses in chicks to sources of unknown growth factors in highly purified diets were no longer obtained in their laboratory. The cessation in growth responses occurred after an extended period of good responses in their four-week tests. Attempts were made to regain the response by applying various types of stress to the birds, such as feeding testosterone, thyroprotein, and cortisone, or changing the protein level of the diet. None of these procedures improved the assay.

However, additional work showed that growth could be depressed by "contaminating" the diets of the chicks with 1 per cent of feces from older birds raised on litter. Under these conditions the feeding of fish solubles (a fishery by-product and a source of unidentified factors) to the feces-supplemented groups resulted in growth differences which were consistently better than those in the uncontaminated groups. After several such experiments the chick battery room itself appeared to have become con-

taminated and growth responses were obtained by adding fish solubles even when feces were not added to the diet.

Barnett and Bird (*loc. cit.*) interpreted their results as being due to changes in microbial population of the environment. This interpretation was based on the report of C. H. Hill and J. W. Kelly (*Poultry Sci.* **33,** *657 (1954)*), which suggested that some connection existed between the intestinal flora and the chick's response to fish meal. Barnett and Bird suggested that certain organisms appeared to be necessary in the environment for the chick's response to unknown factors. Supporting evidence for this theory came from the growth response they secured from feeding 200 mg. of chlortetracycline per kilogram of diet. This high level of antibiotic stimulated growth by itself, so that in the majority of cases the response to fish solubles was either eliminated or greatly reduced.

It is possible that the reason fish solubles increased growth under the contaminated conditions was due to the presence of substances in fish solubles which affected the growth of intestinal microorganisms in a manner favorable to the chick, analogous to the antibiotic effect. This possibility is supported by the researchers' report that adenosine, adenine, and certain related compounds, fed at 150 to 300 mg. per kilogram of diet, stimulated the growth of chicks under similar contaminated conditions (B. D. Barnett, M. Lapidus, H. R. Bird, and F. M. Strong, *Proc. Soc. Exp. Biol. Med.* **92,** *372 (1956)*). Adenosine, adenine, and related compounds, present in fish solubles, are known to be growth factors for certain microorganisms. Growth responses obtained with adenosine in chicks were quite consistent but were not as high as those obtained with fish solubles. The responses were greater in male than in female chicks. The growth-stimulating effect of adenosine must be confirmed, particularly under uncontaminated conditions, before adenosine can be accepted as a true essential nutrient for the chick.

The relationship between antibiotics and unidentified factors appears to differ between laboratories, which also suggests that microbial environments are involved in responses of chicks to sources of unidentified factors. Contrary to the results of Barnett and Bird (*loc. cit.*), E. L. Wisman, C. E. Holmes, and R. W. Engel (*Poultry Sci.* **35,** *457 (1956)*), and Hill and Kelly (*Ibid.* **34,** *1030 (1955)*), have reported that combinations of fish products and antibiotics gave better growth responses in chicks than the sum of the individual effects. Such differences between laboratories might be expected if changes in the microbial flora were involved.

Another method sometimes useful in improving the assay of unidentified growth factors is that of using "depleted" chicks, the offspring of hens fed diets low in unidentified factors. P. E. Waibel, A. B. Morrison, and L. C. Norris (*Ibid.* **34,** *1322 (1955)*) have studied the effectiveness of various practical corn-soybean diets and purified diets for the depletion of unknown factors in hens. It was found that both types of diets resulted in improved responses of the offspring to sources of unidentified factors. This improvement, which was about 10 per cent, would appear to be sufficient to warrant the use of depleted chicks for unidentified factor studies insofar as possible.

The composition of the basal diets used in unidentified factor assays is continually being studied as a means of improving an assay. H. M. Scott, B. C. Johnson, and M. W. Moeller (*Ibid.* **35,** *924 (1956)*) could find no advantage in using sucrose instead of glucose as a source of carbohydrate in purified diets in the assay of the "fish factor" for chicks. This is contrary to previous results of others (*Nutrition Reviews* **14,** *75 (1956)*).

Several recent studies have aided the search for more concentrated sources of

unidentified factors. Egg yolk appears to be a good source of several such factors for the chick, especially the "fish factor" (G. H. Arscott, *Ibid.* **35,** *338 (1956)*; J. H. Hopper, Scott, and Johnson, *Ibid.* **35,** *195 (1956)*). Interest continues in microbial growth products as sources of unidentified factors. Two samples of a Japanese mold culture ("koji" feed) and brewer's yeast were found to be as active as fish meal in promoting growth of chicks fed practical rations (H. Morimoto, S. Ariyoshi, and H. Hoshii *Ibid.* **34,** *1392 (1955)*). Also, J. C. Fritz, F. D. Wharton, R. M. Henley, and R. B. Schoene (*Ibid.* **35,** *552 (1956)*) reported that a fermentation material obtained from cultures of Streptomyces species B-1354 stimulated growth of chicks and turkeys when fed at levels of 0.35 to 1.5 per cent in practical rations. This increased growth was not due to the presence of known vitamins or of antibiotics in the fermentation material.

It is hoped that the improved animal assay procedures for unidentified factors will accelerate evaluation of the tedious processes involved in fractionation and eventual isolation of the active compounds. Although the time for chemical characterization of these factors may not be close at hand, their value is apparent and low-cost concentrates of some of them are already being used in animal rations. The possible application of these unidentified factors to human nutrition must await further study.

ADEQUATE VERSUS OPTIMAL DIET

The experiments of H. C. Sherman and his collaborators, carried on by a generation of scientists upon more than 80 generations of rats, have demonstrated that a diet which is apparently adequate for growth and reproduction may still not be optimal and may be improved by changes in one or more constituents. Thus, apparently normal rats could be raised on a diet consisting of 83 per cent ground whole wheat and 17 per cent whole milk powder, but doubling the amount of milk powder promoted a greater growth-rate and increased longevity (*Nutrition Reviews* **10,** *97 (1952)*). In this case the effect was presumed to be due to the increased amounts of protein, calcium, vitamin A or all three.

In further tests it was found that increased protein added as casein or poultry meat, raising the total protein from 14 to 24 per cent, did indeed increase growth rate (*Nutrition Reviews* **6,** *167 (1948)*; **8,** *229 (1950)*). A similar effect has also been obtained with increased calcium (*Nutrition Reviews* **11,** *177 (1953)*). However, in these experiments, it was found that greater than 0.5 per cent of calcium decreased the number of young successfully weaned, and that the high protein diets resulted in increased mortality within one year and general decreased longevity. The apparent explanation for the protein effect was a calcium deficiency brought about by the increased growth-rate with its accompanying increased demand for that element on a diet which had been barely adequate in calcium at the lower growth-rate.

In a continuation of these experiments, H. C. Sherman *et al.* (*Proc. Nat. Acad. Sci.* **52,** *682 (1946)*) tested the effect of additions of both calcium and protein to the "adequate diet." On the protein-enriched diet (24 per cent protein, 0.2 per cent calcium), males apparently grew more rapidly and showed no decrease in life-span. Females, on the other hand, had shortened life-span and reproductive life. When the calcium content of the diet was raised to 0.4 per cent, both males and females showed more rapid growth and increased length of life, and females had longer reproductive lives. Increase in the calcium to 0.6 per cent did not result in any further benefit to either sex,

indicating that the intermediate amount was optimal for the new growth-rate.

The importance of these experiments in the field of nutrition goes beyond their immediate aim. They show, first, that a diet which is apparently adequate may be improved by certain additions. However, increasing growth-rate with such additions may render the diet inadequate with respect to other constituents and result in a deficiency state.

Other examples of deficiencies brought about by increased growth-rate on a diet rendered more adequate by partial supplementation can be found in the literature. D. J. Mulford and W. H. Griffith (*J. Nutrition* **23,** *91* (*1942*); *Nutrition Reviews* **1,** *168* (*1943*)) demonstrated that the production of a fatty liver by supplementation of a minimal protein diet with cysteine was actually the result of a choline deficiency brought about by the increased growth-rate on the more adequate diet. Similarly, the well-known effect of increased calories as carbohydrate in increasing the thiamine requirement may come under this heading (C. R. Cowgill, *The Vitamin B Requirement of Man, Yale Univ. Press 1934*)).

It is evident from these and similar experiments that under certain circumstances growth-rate alone is not a valid criterion of the adequacy of a diet and that care must be taken in supplementing barely adequate animal or human diets lest a further deficiency be precipitated.

A NEW GROWTH FACTOR

Several years ago it was found that the acetate requirement of certain microorganisms could be replaced by natural materials which did not contain acetate. Subsequent work led to the isolation and characterization of lipoic acid which, in trace amounts, would replace the acetate requirement of some species of bacteria (*Nutrition Reviews* **11,** *59* (*1953*)). This substance exerts a definite biochemical effect in bacteria and in animal tissues. Quite recently another acetate-replacing factor has been isolated and identified chemically, and it also appears to exert a specific biochemical effect in animal tissues.

H. R. Skeggs *et al.* (*J. Bact.* **72,** *519* (*1956*)) observed that cultures of *Lactobacillus acidophilus* would not grow in their basal medium unless either sodium acetate or a sample of distillers' solubles was added. The activity of the distillers' solubles, which is a by-product of the fermentation industry, was much greater than could be accounted for by its acetate content. The basal medium contained lipoic acid, and all the known vitamins were found to be devoid of this acetate-replacing activity. Many natural materials were tested and found to exhibit activity in the assay. These included whole liver, liver extract, crude casein extract, whey and beer. It then appeared that some new factor, capable of replacing acetate for this particular organism, was present in these natural materials.

Steps were next taken to isolate this acetate-replacing factor in pure form. L. D. Wright *et al.* (*J. Am. Chem. Soc.* **78,** *5273* (*1956*)), starting with distillers' solubles, were able to isolate the factor in a fairly pure state. The procedure involved extraction by methanol and chloroform, followed by chromatography on an ion-exchange resin and repeated countercurrent distribution of the active fractions. The success with the isolation procedure was followed by microbiologic assays, the final product being a clear oil which appeared to be essentially pure.

The active material obtained from the isolation procedure was subjected to chemical studies (D. E. Wolf *et al.*, *J. Am. Chem. Soc.* **78,** *4499* (*1956*)) and its structure was

determined. The active compound was β-hydroxy-β-methyl-γ-valerolactone. This compound was synthesized and found to be active in replacing acetate in the microbiologic assay, thus proving its structure.

Of particular interest are the observations relating β-hydroxy-β-methyl-γ-valerolactone (DVA) to cholesterol synthesis (P. A. Tavorimina, M. H. Gibbs, and J. W. Huff, *J. Am. Chem. Soc.* **78,** *4498 (1956)*). Rat liver homogenate was incubated with radioactive acetate, and cholesterol was subsequently isolated. As would be expected, the cholesterol contained radioactivity. The addition of unlabeled DVA depressed the incorporation of acetate into cholesterol. This suggested the possibility that the DVA was being utilized for cholesterol synthesis, thus replacing the acetate. In confirmation of this suggestion it was found that radioactive DVA was incorporated into cholesterol much more rapidly than was acetate. The magnitude of the utilization of DVA for cholesterol biosynthesis suggests that it may be the immediate precursor of cholesterol.

These experiments are of interest from several standpoints. Of particular interest is the participation of DVA in cholesterol biosynthesis. The possibility of synthesizing structural analogs of DVA which may suppress cholesterol biosynthesis, and possibly sterol synthesis in general, has obvious medical application. Also, the possibility always exists that a new factor such as DVA may be useful in the treatment of a specific disease, as in, for example, the use of vitamin B_{12} in pernicious anemia.

METABOLISM OF ESSENTIAL FATTY ACIDS

It is known that the dietary requirement for essential fatty acids in experimental animals can be satisfied by linoleic acid (*Nutrition Reviews* **10,** *154 (1952)*). When an animal is depleted of dietary fat, the amount of linoleic acid (18 carbon atoms, two double bonds), and also of arachidonic acid (20 carbon atoms, four double bonds), in the tissues is decreased. Although the tissues of normal animals contain no significant amounts of fatty acid with three double bonds, such a substance appears during the development of fat deficiency. When linoleic acid is fed to such a depleted animal, the three double-bond fatty acid (not linolenic acid) disappears, and a four double-bond acid (arachidonic) reappears, as well as the two double-bond acid, linoleic. (For a review of the subject *see* "The Vitamins," *Vol. II, p. 268, Academic Press, Inc. (1954)*). From such observations it has been deduced that arachidonic acid is formed in the body from linoleic acid. The origin of the abnormal three double-bond fatty acid, however, has not been understood.

Two papers have recently appeared which elucidate the relationship among these various fatty acids. In the first, J. F. Mead and W. H. Slaton (*J. Biol. Chem.* **219,** *705 (1956)*) report on studies of three double-bond fatty acid from tissues of fat-deficient rats. The compound was isolated and purified and the structure determined. It was found to have the empirical formula $C_{20}H_{34}O_2$ and from spectrophotometric information it was concluded that the three double bonds were separated from each other by $—CH_2$ (methylene) groups. By comparing the hexabromide derivatives of known polyunsaturated acids with that from the unknown acid, and by identifying the carbon fragments obtained after oxidation by ozone, the compound was identified as 5,8,11-eicosatrienoic acid (linolenic acid is 9,12,15-octadecatrienoic acid).

The authors suggest that this three double-bond acid is formed in the deficient animal from arachidonic acid (5,8,11,14-eicosatetraenoic acid) by hydrogenation of one double bond. How the deficient tissues

so modify an essential metabolite remains to be discovered.

In a second study, the same group has shown how arachidonic acid (a 20-carbon acid) is formed in the body from linoleic acid (an 18-carbon acid) (G. Steinberg, W. H. Slaton, D. R. Howton, and J. F. Mead, *J. Biol. Chem.* **220,** *257 (1956)*). Normal rats were fed methyl linoleate labeled in the carboxyl group with radioactive carbon (C^{14}). After four hours the animals were killed, various tissues were removed, and the higher unsaturated fatty acids were isolated as the polybromides. A crude octabromide fraction was obtained. This was debrominated and reduced, and the resulting saturated mixture was chromatographed on a column of siliconized Celite, using mineral oil-acetone-water mixtures to develop the chromatogram. Fractional collections yielded, among other acids, a major fraction of arachidic acid (20 carbon atoms, no double bonds). This acid, which represented the arachidonic acid from the tissues, was degraded one carbon at a time. It was found that of the total C^{14} activity in the isolated arachidic acid, 74.7 per cent was in carbon 1, 0.7 per cent in carbon 2, and 24.5 per cent in carbon 3. There was no activity in the remainder of the molecule.

This showed that the intact linoleic acid molecule became part of arachidonic acid by taking on an acetate molecule. Furthermore, the high activity in carbon 1 showed that most of this acetate was derived from the administered methyl linoleate-C^{14}. It had been shown previously that the carboxyl carbon of linoleic acid is rapidly oxidized when administered to rats (J. F. Mead, G. Steinberg, and D. R. Howton, *J. Biol. Chem.* **205,** *683 (1953)*).

One other saturated fatty acid obtained, behenic acid (22 carbon atoms), also had a high C^{14}-activity, indicating that the carbons in this molecule came from linoleic acid and two active acetates.

As a result of these studies the relationship of linoleic acid to arachidonic acid, and to the previously unidentified trienoic acid, becomes clearer. The extension of these results to experiments with other animals, and to studies at the enzyme level, may eventually reveal the biochemical function of the essential fatty acids.

ALGAE AS A PROTEIN SUPPLEMENT

The greatly expanding world population may result in serious food shortage in many areas (*Nutrition Reviews* **11,** *95 (1953)*; **12,** *111 (1954)*; **13,** *334 (1955)*). Many of the plant proteins are deficient or seriously imbalanced with respect to certain amino acids, and hence are not adequate to completely meet human protein requirements. The problem is especially severe with respect to feeding small children. Many of the plant proteins, particularly those from cereals, are deficient in certain of the essential amino acids and will require supplementation.

One possible source of supplementary protein is the algae. It is possible to grow enormous quantities of the alga *Chlorella* in a relatively small area (*Nutrition Reviews* **11,** *77 (1953)*). It has also been shown that this alga can be added to bread to form an acceptable product (*Ibid.* **13,** *13 (1955)*). Other experiments have shown that *Chlorella* was well utilized for chick growth when it replaced a part of the soybean oil meal in a practical type of ration (*Ibid.* **13,** *45 (1955)*).

Since cereal proteins represent an increasingly large fraction of the world's protein supply, and since large quantities of alga may be produced in small areas, the value of alga as a supplement to cereal protein becomes a matter of considerable practical importance. J. M. Hundley and R. B. Ing (*Science* **124,** *536 (1956)*) have investigated the supplementary value of dried

alga when added to flour and bread diets for rats. In the first experiments weanling rats were fed a basal diet containing 92 per cent white wheat flour and adequate quantities of minerals and vitamins. Rats receiving the basal diet grew poorly. The addition of either 4 per cent dried alga or 0.75 per cent lysine to the basal diet increased the growth rate approximately three-fold. The addition of both supplements resulted in a further increase in growth.

The addition of threonine and lysine to the basal diet gave growth approximately equal to that obtained from lysine and alga. Thus it appeared that the dried alga supplied the threonine required by the rats receiving the flour diet.

Similar results were obtained in a second experiment in which the basal diet was enriched bread. Supplementation with lysine and alga gave growth equivalent to that obtained with lysine and threonine.

It appears likely that in the future as much attention will be paid to amino acid supplementation of bread as is now paid to mineral and vitamin supplementation.

THE REGULATION OF LIPOGENESIS IN ADIPOSE TISSUE

The histologic simplicity of adipose tissue gives no hint of its biochemical complexity and metabolic activity. Mounting biochemical evidence indicates that fat cells are not passive depots for neutral fat but carry on many important processes (*Nutrition Reviews* **13,** *153* (*1955*)). In addition to removing fatty acids from the blood and storing them as triglycerides, fat cells are capable of converting carbohydate to fat. So great is this activity of adipose tissue that it exceeds liver when compared on the basis of protein content. The rate of conversion of carbohydrate to fat within adipose tissue is profoundly influenced by nutrition. During fasting this conversion is greatly retarded. A similar inhibition of fat synthesis occurs in diabetes mellitus and can be restored by insulin administration (*Ibid.* **13,** *115* (*1955*)).

The effect of diet on lipogenesis in adipose tissue has been investigated by F. X. Hausberger and S. W. Milstein (*J. Biol. Chem.* **214,** *483* (*1955*)). Adipose tissue from fasted rats, incubated *in vitro* with radioactive glucose, failed to convert glucose into fatty acids. A similar failure occurred with adipose tissue from rats ingesting a diet containing 60 per cent fat by weight. A progressive increase in fatty acid formation from glucose was found in adipose tissue from rats fed diets containing lesser amounts of fat. The greatest conversion of glucose to fatty acids was found in adipose tissue from rats which had received a diet containing only 0.8 per cent fat and 70 per cent carbohydrate. Pretreatment of the rats ingesting the high fat diet with insulin slightly increased fatty acid synthesis by adipose tissue. The authors feel that the failure of lipogenesis on the high fat, low carbohydrate diet was not the result of insulin lack but was due to an autonomous change in the tissues themselves.

One possible controlling mechanism by which dietary and endocrine factors could regulate lipogenesis has been suggested by S. W. Milstein (*Proc. Soc. Exp. Biol. Med.* **92,** *632* (*1956*)). The oxidation of glucose-1-C^{14} and glucose-6-C^{14} by rat adipose tissue and liver has been measured *in vitro*. By comparing the carbon dioxide formed from the two independently labeled carbons of glucose, an indication can be obtained of the relative activity of the glycolytic cycle and the direct oxidative shunt. By the latter pathway carbon number 1 of the glucose molecule is initially decarboxylated by a triphosphopyridine nucleotide (TPN)-linked reaction. The glucose "shunt" was found to be particularly active in adipose tissue from fed rats. The ratio of radioactivity from C_6:C_1 was 0.18 for adipose tissue as compared to

0.68 for liver. The author did not attempt to determine the absolute contribution of each pathway. Starvation greatly decreased the yield of C^{14} from glucose-1-C^{14} without affecting the oxidation of C_6 in adipose tissue. Similar changes were induced by cortisone administration and alloxan diabetes. The ratios of labeled carbon dioxide from C_6 and C_1 were not changed in liver under identical experimental conditions.

The decrease in activity of the glucose "shunt" mechanism which occurred in diabetic rats was not correctable within three and one-half hours of the administration of insulin but required a longer period of insulin action. After eighteen hours of insulin treatment a high rate of the glucose oxidation by the "shunt" had been restored.

The work of Milstein is of interest in showing an unusually high rate of direct glucose oxidation and also in the suggestion of a possible interrelation of the oxidation of glucose by the "shunt" and the rate of conversion of glucose into fatty acids. R. G. Langdon (*J. Am. Chem. Soc.* **77,** *5190 (1955)*) has reported that reduced triphosphopyridine nucleotide (TPNH) may be required in fatty acid synthesis by a soluble fraction obtained from rat liver. If a similar requirement holds for adipose tissue it is possible that the availability of TPNH from glucose oxidation may limit the rate of fatty acid synthesis. In this way the parallel inhibition of fat synthesis and glucose oxidation by the shunt would not be isolated and unrelated phenomenons. Much more investigative work will be required before this interesting suggestion can be accepted. Moreover, the mechanism by which dietary and endocrine factors could independently control the rate of glucose oxidation by the shunt remains undetermined.

The possible implications of our newer knowledge of adipose tissue metabolism in terms of human disease are intriguing. One may predict that abnormal metabolism of adipose tissue will be shown to be important in the pathogenesis of a number of human diseases. R. D. Lawrence (*Lancet* **I,** *734 (1946)*) has already postulated that in a certain type of diabetes mellitus there is a fundamental defect in lipogenesis in the fat depots.

HORMONAL AND NUTRITIONAL FACTORS INFLUENCING CARDIAC GLYCOGEN

Glycogen in the liver is formed from dietary glucose and other carbohydrate sources and serves as a reservoir of glucose, which is slowly released during the early phase of fasting to maintain the blood sugar. The behavior of cardiac glycogen is paradoxical in that the level rises in normal rats durng fasting. Moreover, the significance of cardiac glycogen is obscure in terms of the metabolism of heart muscle It is known that the heart can function normally during fasting by burning non-carbohydrate nutrients.

J. A. Russell and W. L. Bloom, (*Endocrinology* **58,** *83 (1956)*), have observed that the normal rise in cardiac glycogen with fasting does not occur in hypophysectomized rats. This suggests the importance of the pituitary in determining the level of cardiac glycogen during fasting. Previously, B. A. Illingworth and J. A. Russell, (*Endocrinology* **48,** *423 (1951)*) had shown that growth hormone increased the amount of glycogen in the gastrocnemius, heart, and diaphragm of fasting normal rats and restored to normal the glycogen levels in these tissues of hypophysectomized rats.

Further observations on the effects of nutrition and growth hormone on cardiac glycogen have been made by G. A. Adrouny and J. A. Russell, (*Endocrinology* **59,**

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Further observations on the effects of nutrition and growth hormone on cardiac glycogen have been made by G. A. Adrouny and J. A. Russell, (*Endocrinology* **59,**

241 (*1956*)). Their observations showed the effective dose of growth hormone in hypophysectomized rats to lie between 62.5 and 500 micrograms, and the response to be essentially a linear function of the dose. In normal animals undergoing fasting, the cardiac glycogen rose progressively to reach a plateau after forty-eight hours. Most of the increase in glycogen was in the more labile component, which is extractable by trichloracetic acid.

Since feeding a normal diet to a previously fasted rat causes the cardiac glycogen level to fall within twelve hours to the level resulting from normal feeding, the authors decided to investigate the components of the diet to determine which were most significant in lowering cardiac glycogen. Therefore, fasting normal rats were fed diets consisting of isocaloric quantities of either carbohydrate, protein, or fat. The greatest drop in cardiac glycogen occurred following the carbohydrate feeding. Protein feeding produced a significant, but smaller, fall, and fat feedings were essentially without effect upon cardiac glycogen. The investigators reasoned that the effectiveness of carbohydrate feeding might have resulted either from a decrease in secretion of pituitary factor, or from an inhibition of the activity of circulating hormone.

The action of growth hormone on cardiac glycogen was shown to be unimpaired by carbohydrate feeding. This was done first by fasting rats, and then by giving them graded doses of growth hormone after the restoration of food. The increment in total glycogen twelve hours after the administration of growth hormone was not significantly different from that which was found in the fasting rats. This would indicate that growth hormone could counteract the glycogen-lowering effect of food. Some doubt on this point must remain because the authors comment that when the period of growth hormone action was prolonged to twenty-four hours, little increase in cardiac glycogen was observed.

The observations which have been discussed provide support for the hypothesis that the rise in cardiac glycogen which occurs with fasting, at least in the rat, is related to the release of a pituitary factor. Although growth hormone preparations possess the ability to raise the level of cardiac glycogen, there is some uncertainty concerning the identity of this activity. No good, direct evidence exists which shows that the level of growth hormone fluctuates inversely with food intake. It is difficult to see how such a pattern of release could be helpful in achieving the primary action of growth hormone, namely increased growth. A clearer understanding of the effect of nutritional factors on pituitary function is urgently needed.

THE GASTRIC JUICE AND THE INTRINSIC FACTOR OF PERNICIOUS ANEMIA

The long unanswered nutritional riddle of the nature and mechanism of the action of Castle's intrinsic factor appears to be a step nearer solution as a result of a recent intensive study of human gastric juice by R. Gräsbeck (*Acta. med. Scandinav.* **154,** *Suppl. No. 314, 1–85 (1956)*). Although there appears to be increasing evidence that the intrinsic factor is mucoprotein in nature, the final steps of identification and chemical characterization are not yet complete (G. B. J. Glass, *Rev. Hematol.* **10,** *137 (1955)*). In his study, Gräsbeck has applied himself particularly to a study of the relationship between vitamin B_{12}-binding principle isolated from human gastric juice and the intrinsic factor.

Using starch electrophoresis to fractionate

the colloidal components of gastric juice, Gräsbeck found two components of human gastric juice which bound vitamin B_{12}. Since the faster migrating and more heat-stable component varied considerably from experiment to experiment it was suspected of being either an extra-gastric contaminant or an autodigestion product resulting from the action of pepsin on the slower moving component.

By means of similar techniques saliva was found to have only one fraction capable of binding vitamin B_{12} and this fraction was not identical with either of the vitamin B_{12}-binding components from gastric juice. Furthermore, gastric juice was found to inactivate the salivary binding component. These and other considerations led Gräsbeck to conclude that the salivary factor which binds vitamin B_{12} is not identical with either of the gastric fractions which demonstrate this capacity. Further investigation indicated that vitamin B_{12} bound to gastric juice tended to dissociate at certain pH values. This was abolished by alkaline inactivation of the pepsin and offered additional evidence that autodigestion by pepsin was responsible for the one of the two components of gastric juice which bound vitamin B_{12}. When pepsin was inactivated at pH 10 prior to electrophoretic analysis of the gastric juice only one vitamin B_{12}-binding component was found.

Additional analysis of this vitamin B_{12}-binding component indicated that it was stable to treatment with alkali, that it was nondialyzable, heat-labile, and slowly digestible with pepsin. Physicochemical characteristics included an electrophoretic mobility of -3×10^{-5} (pH8) and a diffusion constant indicating a molecular weight of 70,000.

When intrinsic factor activity of different parts of the electrophoretically fractionated human gastric juice was determined, activity was found only in the vitamin B_{12}-binding fraction. For each fraction, radioactive vitamin B_{12} and the urinary excretion test were utilized to assay intrinsic factor activity in 3 pernicious anemia patients. Comparison of this method of assay with the microbial growth inhibition technique indicated that the latter was inferior in the assay of intrinsic factor.

On the basis of his study Gräsbeck suggests that, within certain limits, the B_{12}-binding power of fractions of human gastric juice may be used as an *in vitro* method of assay for intrinsic factor. On the other hand he points out that intrinsic factor may have other important physiological capacities in addition to binding vitamin B_{12}. He also noted that extra-gastric material having no intrinsic factor activity can still possess vitamin B_{12}-binding power. He was not, however, able to find evidence of intrinsic factor activity in the fraction containing glandular mucoprotein (the leading anodic peak on electrophoretic analysis), as had been reported by others (G. B. J. Glass, *Rev. Hematol.* **10,** *137* (*1955*)). Of considerable interest is the apparent physicochemical identity between active intrinsic factor components from hog stomach and those which Gräsbeck has isolated from human gastric juice.

Altogether the author presents a convincing thesis for the identity of the B_{12}-combining component of gastric juice and intrinsic factor. Allowing for the complication of autodigestion in the experiments of others, most of the apparent incongruities among the data of various investigators can be explained. Although more work will have to be done, it appears that at long last an *in vitro* method for assay of intrinsic factor may be available. The time may also be near when an understanding of the nature and mechanism of action of Castle's intrinsic factor will be clarified. This should aid considerably in the overall understanding of how vitamin B_{12} is absorbed from the gastrointestinal tract and of how the various deficiency states develop which lead to macrocytic anemias.

CALCIFICATION OF FETAL BONE

Relatively little attention has been given by nutritionists to the deposition of calcium in bones of the mammalian fetus. This subject has, however, been recently reviewed by C. L. Comar (*Ann. N. Y. Acad. Sci.* **64,** *281* (*1956*)) with emphasis on the role of radioactive tracers in such studies.

Which is the direct source of fetal calcium, the diet or the mother's skeleton? At what fetal age does calcification proceed most rapidly? Are there differences in rates of calcium deposition of the various types or parts of bones? Are there significant differences in deposition among the fetuses of various mammals? Is placental transfer of calcium passive or active? These questions have been solved, at least partially, by means of the radioactive tracer technique.

An answer to the first question has been provided by studies with strontium^{90} (C. L. Comar, I. B. Whitney and F. W. Lengeman, *Proc. Soc. Exp. Biol. Med.* **88,** *232* (*1955*)). Female rats were raised on a diet containing a constant ratio of strontium^{90} to calcium. When these rats gave birth to offspring, it was found by analysis of specific activity (Sr^{90}/Ca) that approximately 30 per cent of the calcium in the bones of the fetus was derived from the skeleton of the mother. This was calculated by the usual methods of isotope dilution, the specific activity of the dam's bones prior to conception being determined by the sacrifice of a suitable control group. Periodicity of feeding and rate of absorption are thought to govern to a large extent whether fetal calcium is derived more from the maternal skeleton or from the diet. Although there is no direct evidence for such a cycle in mammals, it has recently been observed to obtain for the egg shells of birds (C. Tyler, *J. Sci. Food Agr.* **5,** *335* (*1954*)).

Many studies have been carried out which show that the last calcium fixed in the mother's bones is the first to be removed for transfer to the fetus. This has been determined by administering radiocalcium to the dam before calcification has started in the fetus so that the radiocalcium becomes sequestered in the mother's bone. Bones of the developing fetus show a higher specific activity (Ca^{45}/Ca) than does the skeleton of the dam. This means that some of the last calcium laid down in the mother's bones was mobilized and transferred to the fetus preferentially as compared with the rest of the maternal calcium.

It was noted that little calcium is deposited in the fetus during the first trisemester of pregnancy and that the greatest accumulation occurs during the last trisemester (M. P. Plumlee, S. L. Hansard, C. L. Comar and W. M. Beeson, *Am. J. Physiol.* **171,** *678* (*1952*)). When comparison is made among rat, bovine and human fetuses, it is found that the rate of increase is fastest for the rat, slower for the bovine, and slowest for the human fetus.

Differences in sites of deposition are observed at different stages of development. Thus, in bovine fetuses of 3½ months, more Ca^{45} appeared in the shafts than in the epiphyses. This situation was reversed at 6 and 7½ months. For all fetal ages a higher turnover rate of Ca^{45} was observed in the epiphysal bone than in the shaft areas. A decrease in specific activity of fetal bone with age was noted. This was due primarily to the fact that the normal calcium content of fetal bone increases with age.

Placental transfer of calcium ions is thought to be similar to the rate of transfer across this membrane of inorganic phosphate (W. S. Wilde, D. B. Cowie and L. B. Flexner, *Am. J. Physiol.* **147,** *360* (*1946*)). Some general conclusions from a series of determinations were as follows: Inorganic phosphate is supplied to guinea pig fetuses in about the quantities required for growth. For example, it was shown using P^{32} that a 105 gm. fetus received 1.8 mg. phosphorus per hour as compared with an actual phos-

phorus retention during growth of 1.5 to 2.2 mg. per hour. This is unlike sodium and water which are supplied to the fetus in amounts many times those required for growth. The phosphorus retained by the fetuses per hour was about 4 times the total inorganic phosphorus retained in the maternal plasma.

An indication of the rapidity of placental transport of calcium in the rat is available from the work of M. P. Plumlee (*loc. cit.*). Within thirty minutes after oral administration, approximately 63 per cent of the Ca^{45} had been absorbed by the mother and 14 per cent of the dose was already transferred to the fetus. Within one hour, the fetuses had incorporated the maximum quantity of radioisotope.

Concerning the mechanism of placental transfer, it is obvious that fetal requirement is a governing factor since the calcium content of the maternal plasma often falls during the latter term of pregnancy. This cannot be explained by a drop in the level of fetal plasma calcium since this is higher than the maternal plasma calcium, Thus, "active transport" is probably involved. C. L. Comar suggests that the high arginine content of the placenta may play a role, since it has recently been found that arginine and lysine are quite effective in promoting calcium absorption from the digestive tract.

NOTES

Fat Absorption in the Small Intestine

Although the small intestine is the principal site of fat absorption, few attempts (*see*, A. C. Frazer, *J. Physiol.* **102,** *306* (*1943*); A. J. Kremen, J. H. Linner, and C. H. Nelson, *Ann. Surg.* **140,** *439* (*1954*)) have been made to localize more exactly the site of optimal absorption. Recent studies by J. A. Benson, G. N. Chandler, F. E. Vansteenhuyse, and J. O. Gagnon (*Gastroenterology* **30,** *53* (*1956*)) indicate that in the rat optimal absorption of olive oil occurs in the third quarter of the small intestine. Rats were fasted for forty-eight hours (but allowed free access to water), then gavaged (fed by stomach tube) with a measured test meal of radioactive iodine (I^{131})-labeled olive oil, and killed after varying periods of time up to six hours. Thereafter, measurements of the radioactivity of 20 segments of equal length of intestine (from pylorus to cecum) were made. The results were expressed as counts per second, corrected for decay for the time elapsed since administration of the olive oil. In order to correlate results from individual rats, the radioactivity of each segment was calculated as a percentage of the total small intestine activity for each rat.

Using this technic, Benson *et al.* found that maximal absorption of olive oil occurred in the third quarter of the length of the small intestine, regardless of the distance of this area from the pylorus. The authors believe that the radioactivity measurement was a function of absorbed fat present in the intestinal wall because of (1) the disproportion between intraluminal contents and tissue radioactivities, (2) the negligible radioactivity recovered in the intestinal wall after gavaging aqueous unbound I^{131} with or without unlabeled olive oil, and (3) the fact that "dialysis" did not affect the reproducibility of the principal peak. Why the third quarter of the rat small intestine should be the site of optimal absorption of olive oil is not known, but the authors suggest that "in all probability it reflects a heightened capacity for cell work, perhaps by cellular enzyme systems."

These results will be of interest to those engaged in studies of fat absorption, and may eventually prove important in determining the relative merits of the "lipolytic" and "partition" hypotheses of fat absorption.

Calcium Metabolism and Surgical Trauma

Because of some unreported findings regarding the appearance of hypocalcemia after trauma and its possible clinical implications, G. H. Peddie, C. L. Spurr, and J. M. Howard (*Ann. Surg.* **143,** *452 (1956)*) felt it important to investigate calcium and phosphorus balances in the patient undergoing surgery. In the study the calcium intake was evidently at a very high level (2.5 g. daily) and this was abruptly decreased to zero at the time of surgery and for a period, usually about five days, thereafter. The importance of undertaking prolonged balance periods does not seem to have been considered, as the negative calcium balances are suggestively correlated with surgery rather than with the abrupt changes of calcium intake and subsequent readjustments.

Occasionally the values noted are hardly credible; for example, positive calcium balances of 1.0 g. daily are cited, and the occurrence of negative balances postoperatively with "maximum" excretion of 2.5 g. per day. In part, some of these errors may be analytic, but analysis of this last-quoted statement reveals that the previous period of high calcium intake was reflected in the next fecal collection, although this fact was not appreciated in the report.

Thus, the authors generally chose to investigate metabolic parameters which do not mirror the rapid metabolic changes occurring after surgery.

There are a number of less serious criticisms. The metabolic charts are not patterned after those of E. C. Reifenstein, F. Albright, and S. L. Wells (*J. Clin. Endocrinol.* **5,** *367 (1945)*), although such is suggested by the authors. The statement is made that the "excretion (of calcium) is chiefly by way of the feces and seldom varies more than 50 milligrams from the amount ingested." This is obviously consistent with the presumed positive calcium balances of 1.0 g. and with data from the literature. Another statement, with which a number of bone physiologists would disagree is, "This entire mechanism of calcium shift is under the influence of the parathyroid glands."

Without belaboring the point, it appears that considerable work has been done in an attempt to understand a problem in a setting difficult of analysis. A lack of appreciation of some of the pitfalls of balance studies concerning calcium has led to the recording of meaningless data.

Effects of Prolonged Cortisone Therapy on Growth

The antianabolic effects of cortisone and the decreased stature of children with Cushing's syndrome indicate that steroids of the cortisone type may inhibit growth. It therefore seemed pertinent to examine the effects of long-continued cortisone therapy on growing children (F. M. Blodgett and co-workers, *New Engl. J. Med.* **254,** *636 (1956)*).

The children studied were those being treated with cortisone for adrenal virilism, hypopituitarism, Addison's disease or allergy. Heights, weights, X-rays of bone and pertinent chemical determinations were obtained at frequent intervals. The diet was given ad libitum, and only 3 children received supplemental potassium chloride.

The rate of growth and the rate of skeletal maturation of the 11 patients with adrenocortical virilism were correlated with the dose of cortisone. In these children about 35 mg. of cortisone per square meter of body surface area were necessary to bring the growth rate to the average expected for children of the same sex and age. At higher dose levels there was a tendency for growth rates to be suppressed to levels below normal.

In 2 children with Addison's disease and in 20 children with allergies, cortisone doses of 4 to 20 mg. per square meter of body surface either did not alter the growth rate or slightly increased it. This same dose range, however, demonstrated a marked

growth suppressing effect in the 3 patients with hypopituitarism.

Several illustrations are presented indicating that a growth spurt will result when the cortisone is decreased below the growth-suppressive dosage. It was not necessary to stop administration of the steroid in order to demonstrate this effect.

It was generally noted that when more than 50 mg. of cortisone per square meter were administered, there was a tendency for weight gain due to increased appetite. This was also observed in the hypopituitary patients on doses as low as 10 to 20 mg. of cortisone per square meter of body surface area. Continuation of cortisone administration at these levels led to stigmata of Cushing's syndrome.

This study demonstrates several points. First, it is possible to give cortisone to children uninterruptedly for long periods of time without altering the normal growth rate. Second, when the cortisone dosage leads to growth suppression, it should be decreased at intervals to allow compensatory growth spurts. Third, the hypopituitary patient is more sensitive to lower doses of cortisone than any other group. This may be due to the concomitant hypofunction of other endocrine glands known to be important in the growth response, the thyroid and gonads.

Cholesterol Absorption in the Rabbit

Most studies of cholesterol absorption have generally been carried out in animals which have been on high cholesterol diets for several weeks. In the experiments of R. Wolf and J. J. Brignon (*Bull. Soc. chim. biol.* **38,** *99* (*1956*)) however, it was found that twenty-four hours after ingesting 0.25 to 0.50 g. of cholesterol in cottonseed oil, rabbits had a serum cholesterol concentration approximately double their normal postabsorptive level.

A study of the effect of the vehicle on the extent of absorption as measured by the twenty-four hour level revealed that, as had been found previously for rats (*Nutrition Reviews* **13,** *246* (*1955*)), oleic acid enhanced the rate of cholesterol absorption. Soy sterols, on the other hand, if given in six-fold excess over the cholesterol, produced complete inhibition of absorption. At levels lower than four-fold, the effects were variable. These results agree quantitatively with those of O. J. Pollack (*Circulation* **1,** *696* (*1953*)) whose experiments, however, extended over a fourteen-day period.

These experiments show that in the rabbit, at least, the effects of a variety of factors influencing the absorption of cholesterol and, indirectly, its concentration in the blood, can be determined accurately and rapidly. If such a rapid test could be applied to human subjects it would be very useful in assessing the effects of many dietary agents on serum cholesterol levels.

Clinical Usefulness of Plasma Pepsinogen Assays

In an effort to evaluate the status of gastric secretory activity in man without resorting to passage of a stomach tube, the utility of the plasma pepsinogen assay has been investigated. The initial survey of plasma pepsinogen levels demonstrated a positive correlation between increased plasma pepsinogen concentration and peptic ulcer. The levels were decreased after gastrectomy or in the presence of an atrophic gastric mucosa (*Nutrition Reviews* **11,** *28* (*1953*)).

H. M. Spiro, A. E. Ryan, and C. M. Jones (*New Engl. J. Med.* **253,** *261* (*1955*)) used essentially the same assay method (*Nutrition Reviews, loc. cit.*), which depends upon the enzymatic digestion of hemoglobin, and found that plasma pepsinogen levels are constant from day to day. C. S. Hoar and J. R. Browning (*New Engl. J. Med.* **255,** *153* (*1956*)) confirmed the day-to-day constancy of the enzyme level and noted that there was little variation throughout the day and that meals exerted no perceptible influence on the plasma levels. These ob-

servations imply that a constant small fraction of the pepsinogen elaborated by the gastric chief cells enters the blood stream directly, as the elevated intragastric levels of pepsin following a meal are not reflected in the plasma levels.

Both teams of investigators established normal values for their laboratories. Since the pH of the digestion mixtures in the studies varied between the two laboratories, comparison of the reported units cannot be made, but qualitative results were generally in agreement. Patients with duodenal ulcer had significantly higher levels of plasma pepsinogen than the control groups. Spiro *et al.*, in a small series of 20 patients with gastric ulcer, demonstrated a tendency for the plasma pepsinogen levels to be elevated, although there was a large overlap with the normal subjects. Hoar and Browning, however, found that these levels in 15 patients with gastric ulcer were the same as those found in patients with duodenal ulcer.

Patients with carcinoma of the stomach showed a wide range of plasma pepsinogen levels, from high to very low (Spiro *et al.*, *loc. cit.*). In 5 patients examined by Hoar and Browning, plasma pepsinogen was significantly lower than normal levels. This point needs, and is worthy of, further study.

Hoar and Browning determined the plasma pepsinogen level in 33 patients with pernicious anemia. The values were consistently low and did not differ from the values found after total gastrectomy. ACTH, cortisone and hydrocortisone did not exert any effect on plasma pepsinogen in a small group of patients.

These data suggest that a plasma pepsinogen assay would be useful in the differential diagnosis of upper gastrointestinal bleeding, since a low value would argue against the diagnosis of peptic ulcer. Its worth in signaling the presence of gastric cancer is debatable at present, but investigations in this area will surely be prosecuted further.

Recent Nutrition Books

The Fat of the Land. Enlarged edition of *Not by Bread Alone*. Vilhjalmur Stefansson with comment by Frederick J. Stare, and Paul Dudley White. Macmillan Co., New York, 1956. Pp. 339. Price $5.00.

Body Measurements and Human Nutrition. Edited by Josef Brozek. Wayne University Press, Detroit 1, Michigan. Pp. 164.

Plant Physiology. Meirion Thomas, with the collaboration of S. L. Ranson and J. A. Richardson. 4th edition, revised. Published by Philosophical Library, Inc., 15 East 40th Street, New York 16, N. Y. Pp. 655; 89 illustrations. Price: $12.00.

Foundations of Nutrition. Clara Mae Taylor, Grace MacLeod, and Mary Swartz Rose. 5th edition. Published by The Macmillan Company, New York, 1956. Pp. 620. Price: $6.00.

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